From the INTERNATIONAL BUREAU

PCT NOTIFICATION OF THE RECORDING OF A CHANGE (PCT Rule 92bis.1 and Administrative Instructions, Section 422) Date of mailing (day/month/year) 07 September 1999 (07.09.99) Applicant's or agent's file reference DUNW/P20111PC	MILES, John Eric Potter Clarkson Park View House 58 The Ropewalk Nottingham NG1 5DD ROYAUME-UNI
International application No. PCT/GB98/03766	International filing date (day/month/year) 15 December 1998 (15.12.98)
1. The following indications appeared on record concerning: the applicant the inventor X Name and Address BASSETT, Richard Eric Potter Clarkson Park View House 58 The Ropewalk Nottingham NG1 5DD United Kingdom	the agent the common representative State of Nationality State of Residence Telephone No. 0115 955 2211 Facsimile No. 0115 955 2201 Teleprinter No.
2. The International Bureau hereby notifies the applicant that the the person X the name the add	
Name and Address MILES, John Eric Potter Clarkson Park View House 58 The Ropewalk Nottingham NG1 5DD United Kingdom	State of Nationality Telephone No. 0115 955 2211 Facsimile No. 0115 955 2201 Teleprinter No.
3. Further observations, if necessary:	
4. A copy of this notification has been sent to: X the receiving Office the International Searching Authority X the International Preliminary Examining Authority	the designated Offices concerned X the elected Offices concerned other:
The International Bureau of WIPO 34, chemin des Colombettes	Authorized officer Patricia Gonzalez

Telephone No.: (41-22) 338.83.38

Form PCT/IB/306 (March 1994)

Facsimile No.: (41-22) 740.14.35

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NOTIFICATION OF ELECTION

(PCT Rule 61.2)

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Assistant Commissioner for Patents United States Patent and Trademark Office Box PCT Washington, D.C.20231 ÉTATS-UNIS D'AMÉRIQUE

Date of mailing (day/month/year)
07 September 1999 (07.09.99)

International application No.
PCT/GB98/03766

International filing date (day/month/year)
15 December 1998 (15.12.98)

Applicant
SCHOR, Seth, Lawrence et al

1.	The designated Office is hereby notified of its election made:
	X in the demand filed with the International Preliminary Examining Authority on:
	16 July 1999 (16.07.99)
	in a notice effecting later election filed with the International Bureau on:
2.	The election X was
	was not
	made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland

Authorized officer

Patricia Gonzalez

Telephone No.: (41-22) 338.83.38

Facsimile No.: (41-22) 740.14.35

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WORLD INTELLECTUAL PROPERTY ORGANIZATION International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6: C12N 15/12, 5/10, 15/63, C07K 14/78, 16/18, C12Q 1/68, G01N 33/574, A61K 38/39

(11) International Publication Number:

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24 June 1999 (24.06.99)

(21) International Application Number:

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A1

(22) International Filing Date:

15 December 1998 (15.12.98)

(30) Priority Data:

9726539.1

16 December 1997 (16.12.97) GB

(71) Applicant (for all designated States except US): UNIVERSITY OF DUNDEE [GB/GB]; 11 Perth Road, Dundee DD1 4HN (GB).

(72) Inventors: and

(75) Inventors/Applicants (for US only): SCHOR, Seth, Lawrence [GB/GB]; Unit of Cell and Molecular Biology, The Dental School, University of Dundee, Dundee DD1 4HR (GB). SCHOR, Ana, Maria [ES/GB]; Unit of Cell and Molecular Biology, The Dental School, University of Dundee, Dundee DD1 4HR (GB).

(74) Agent: BASSETT, Richard; Eric Potter Clarkson, Park View House, 58 The Ropewalk, Nottingham NG1 5DD (GB).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published

With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: POLYPEPTIDES, POLYNUCLEOTIDES AND USES THEREOF

STOCCTORGO ACAGCOSTOC CCTCCACGOO AGCCTCGAAG AGCAAGAGGC 151 AGGCTCAGCA AATOUTTCAG COCCAGTCCC COUTOGCTGT CAGTCAAAGC 201 AAGCCCGGT: GTTATGACAA TGGAAAACAC TATCAGATAA ATCAACAGTG 251 GGAGCGGACC TACCTROOCA ASSOCUTTOOT TROTACTIOT TATOGRAPOAN OCCURAGO TT TRACTOCCIAG AGTRAACCEG AAGCTGAAGA GACTTGCTTT GAATI AGCTO TACCATCOCA AACCOCTOCC ATGAAGOOGG TCAGTCCTAC ANGUITOUTO ACACCTOGAG GAGACCACAT GAGACTOUTO GITACATOTT ACCUPATION TOTAL TOTAL ATTOCALANCE ACCUPATION OF THE PROPERTY ATTOCALANCE ACCUPATION ACC TERCTORIORA GEOTETTORE CREGOTOCTO GUACTICCEA TORGOTOCOGA CAMACUTORS AGRAGOCOTA COMAGGOTOG ATGATOGTAG ATTGTACTTG ACCIATCAGGA CACAAGGACA TECTATAGAA TEGGAGACAC CEGGAGCAAG AAGGATAATC GAGGAAACCT OCTOCAGTOC ATCTOCACAG GCAACGGCCG 151 AGGAGAGTOG AAGTOTGAGA GGCACACCTC TOTGCAGACC ACATCGAGCU 901 GATCTOGCCC CTTCACCGAT OFTCGTGCAG CTGTTTACCA ACCGCAGCCT 951 CACCCCCAGC CTCCTCCCTA TOGCCACTGT GTCACAGACA GTGGTGTGGT 1001 CTACTOTGTO GOGATOCAGT GOGTGAAGAC ACAAGGAAAT AAGGAAATGC 1051 TTTOCACOTO CCTOGOCAAC OGAGTCAGCT OCCAAGAGAC AGCTGTAACC

1101 CAGACTIACO GIOGOAACTE AAATOCAGAG CONTUTOTE TROCATICAE
1151 CINCAACOGAE AGGACOGACA GEACHACTET GAATTATOAG CAGGACCAGA
1201 AATACTETT CTOCACAGAG CACACTOTIT TOUTTCACAC TOGAGGAGGA
1201 AATTOCACAG GIOCOTTOTO CONCITECCE TITOCATACAE ACACACACACA
1301 TITACACTORT TOCACTICTO AGGOCAGAAG AGACAACATO AATTOGOTTOT
1301 OCTOCCCACO AGGAALTETO CACACACAA AGTITOGOTT CTOCCCCATO
1401 OCTOCCCACO AGGAALTETO CACACACAA GATTAGOGTICA TOTACCOCAT
1401 OCTOCCCACO AGGAALTETO CACACACAA GATTAGATTOC ATGAGGACA
1501 COUTOTOTO GAATAGATOTO COGGALTOGA CATOCATTOC CIACTOCAG
1501 COTOTOCACA AGGACATOTO TOATGACAT CACTOCACAA TOCCAGGAT
1401 ATTOCACAAG COTCATGAAG AGGGGCACA COCTUAACTOT ACATOCATCO
1401 OTCAGGGTCO GGACGATTTA TCAAATTOGA GATTCATOGO AGAAGTATOT
1701 TCAGAGACTO GGACGATTTA TCAAATTOGA GATTCATOGO AGAAGTATOT
1701 COCATOCCA ACCTITACAG ACCTATOCAA GCTCAACTOCA CTCTOTCGAA
1801 OGCATOCCA ACCTTTACAG ACCTATOCAA GCTCAACTOCT CACACCAGTO
1801 GAATOCACCA CAGCCACCAC AACCTTOCAA GTACATTCCC ACGTCGGAGA
1801 CAATTCCACAC CAGCCACCAC AACCTTOCAA GTACATTCCC CCACCCAGTO
1901 CAATTCCACT GUTTCCTTT TTTCCCACCT TITOAGCCAA CAACTCTGAT
1901 CAATTCCACT GUTTCCTTT TTTCCCACCT TITOAGCCAA CAACTCTGAT
1901 CAATTCCACT GUTTCCTTTT TTTCCCACCT TITOAGCCAA CAACTCTGAT
1901 CAATTCCACT GUTTCCTTTT TTTCCCACCT TITOAGCCAA CAACTCTGAT
1901 TAACATATCC TATAGCATT ACTATATTTT TITTAGTGAAC CAACTCTGAT
1901 TAACATATCC TATAGCATT ACTATATTTT TITTAGTGAAC AAACATATCT
1901 TAACATATCA TATAGAATTA ACTATATTTT TITTAGTGAAC AAACATATCT
1901 TAACATATTA AATTGACTTT ACTATATTTT TITTAGTGAACATATCT
1901 TAACATATTA AATTGACTTT TAGATTTA TATAGTGAACATATCT
1901 TAACATATTA AATTGACTTT TAGATTTA TATAGTGAACATATCT
1901 TAACATATTA AATTG

(57) Abstract

A recombinant polynucleotide encoding migrating stimulating factor (MSF) or variants or fragments or derivatives or fusions thereof distinguish polynucleotides which encode MSF or fibronectin. These reagents are believed to be useful in, for example, diagnosing cancer.



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16 December 1997 (16.12.97) 9B

(71) Applicant (for all designated States except US): UNIVERSITY OF DUNDEE [GB/GB]; 11 Perth Road, Dundee DD1 4HN (GB).

(72) Inventors; and

(75) Inventors/Applicants (for US only): [SCHOR, Seth, Lawrence [GB/GB]; Unit of Cell and Molecular Biology, The Dental School, University of Dundee, Dundee DD1 4HR (GB).

SCHOR, Ana, Maria [ES/GB]; Unit of Cell and Molecular Biology, The Dental School, University of Dundee, Dundee DD1 4HR (GB).

(74) Agent: BASSETT, Richard; Eric Potter Clarkson, Park View House, 58 The Ropewalk, Nottingham NG1 5DD (GB). (81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published

With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: POLYPEPTIDES, POLYNUCLEOTIDES AND USES THEREOF

1 CARACTION COCKACTICS CTCCCONTOC GOOGNETCTCT CCCCCACCOT 51 CICAACATOC TIMOSOUTCE GOOGCCCOOG CTUCTUCTOC TOGCCOTCCA 101 GTOCCTOGGG ACAGCGGTGC CCTCCACGGG AGCCTCGAAG AGCAAGAGGC 151 MOSTICAGEA BATOSTICAS COCCASTOCC CONTOSCIST CASTONARSC 201 ARGEOGOGIT GITATGACAA TOGGAAACAC TATCAGATAA ATCAACAGTG 251 GOLDCOOLCC TACCTROOCA AFOCOTTOOT TROTACTTOT TATOGRAGUA 301 OCCORDOTYT TAACTGODAG AGTRAACCTG ARGCTGANGA GACTTGCTTT GACAAGTACA CTGGGAACAC TEACCOAGTO GGTGACACTT ATGAGCGTCC 351 THANGACTICS ATGRICTORG ACTURACCIO CATOROGOCT GEOCGAGGGA CHATRAGETG TACCATCOCA ANCCOCTOCC ATGANGGOOG TCAGTCCTAC AMERITAGING ACACCITOGAG GAGACCACAT GAGACTOGING GITACATOTT ACASTOTORO TOTOTTOSTA ATOCIMANOS MUNTOSACO TOCHASCOCA 601 TAGCTORGRA GEOTTTTGAT CATGCTGCTG GGACTTCCTA TOTOGTCGGA 651 GARACUTORS AGRAGOCOTA CORROCTOS ATGRICOTAS ATTOTACTIO 761 CCTGGGGGGA GGCAGCGGAC GCATCACTTG CACTTCTAGA AATAGATGCA 851 AGGRONOTOG AMOTOTOMOM OGCHCACCTC TOTOCAGACC ACATCORGCO 901 GATCHOGCCC CTTCACCOAT GTTCGTGCAG CTGTTTACCA ACCGCAGCCT 1001 CTRCTCTUTG GGGATGCAGT GGCTGAAGAC ACAAGGAAAT AAGCAAATGC TITICACUTG CCTOGGCAAC GGAGTCAGCT GCCAAGAGAC AGCTGTAACC

1101 CAGACTTAGG GTGGCAACTC AAATGGAGAG GCATGTGTCT TACCATTGAG 1151 CTACAACGAC AGGACGGACA GCACAACTEC GAATTATGAG CAGGACGAG 1201 ANTACYCTET CTOCHCHOIC CACACTOTYT TOOTYCAGAC TOGAG 1251 AATTOCAATO GEOCCTIGEO CONCTEGCC TECCTATACA ACAACCACAA 1301 TEACHCRAFT TOCHCTICTG AGGOCAGAAG AGACAACATG AAGTGGTGT 1351 GGACCACACA GAACTATGAT GCCGACCAGA AGTTTGGGTT CTGCCCCCATG 1401 GCTGCCCACG AGGARATCTG CACAACGAT GAAGGGGTCA TOTACCGCAT 1451 TOURGATCHG TOOGRATANCE ASCATORCRY GESTCHCATG ATGRESTICAL 1501 CUTOTUTTOG GRATOOTCUT GOOGRAFOGA CATGCATTOC CTACTCOCAG 1551 CTTCOMMEC ACTOCATION TOATGACANC ACTUACHANG TORACGACAC 1601 ATTOCKOMO COTCRIGAGO AGGOGGACAT GCTGAACTOT ACATGCTTCG 1651 OTCHOOGTCG GOGCROUTOG ARGTOTGATC CCGTCGACCA ATGCCAGGAT 1701 YCAGAGACTG GGACGTITTA TCANATTGGA GATTCATGGG AGAAGTATGT 1751 GCATGOTGTC AGATACCAGT GCTACTGCTA TGGCGGTGGC ATTGGGGAG 1801 GOCATTOCCA ACCTITACAG ACCTATOCAA GCTCAAGTGG TCCTGTCCAA 1851 GIATTIATCA CTGAGACTCC GAGTGAGCCC AACTCCCACC CCATCCAGTG 1901 GAATGCACCA CAGCCATCTC ACATTTCCAA GTACATTCTC AGGTGGAGAC CHARGE THE COURCEAGE ASCUTIONAL ACTUATION CHARCETER CHATTCHIAT GOTTTCTTTT TTTGCCAGCT TTTGAGCCIA CAACTCHGA 2051 TARCTATICC TATAGCATTS ACTAINTING THTAGTGARC ARACMATATE 2101 TODICANTA ANTOACTIG TACACTGARA MARARARA AMARARA

(57) Abstract

A recombinant polynucleotide encoding migrating stimulating factor (MSF) or variants or fragments or derivatives or fusions thereof or fusions of said variants or fragments or derivatives. Reagents are disclosed which can distinguish MSF and fibronectin, and which can distinguish polynucleotides which encode MSF or fibronectin. These reagents are believed to be useful in, for example, diagnosing cancer. MSF or variants or fragments or derivatives or fusions thereof, or fusions of said variants or fusions or derivatives, are useful in modulating cell migration and in wound healing.

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DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

Inter onal Application No

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/12 C12N5/10 C07K14/78 C07K16/18 C12N15/63A61K38/39 G01N33/574 C1201/68According to International Patent Classification (IPC) or to both national classification and IPC **B. FIELDS SEARCHED** Minimum documentation searched (classification system followed by classification symbols) IPC 6 C07K Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practical, search terms used) C. DOCUMENTS CONSIDERED TO BE RELEVANT Relevant to claim No. Citation of document, with indication, where appropriate, of the relevant passages WO 94 16085 A (ZYMOGENETICS INC) 1-3, X 6-13,2721 July 1994 29,53-57 4,5,51, see abstract; claims Α 52 see page 2, line 30 - page 4, line 8 9-18,25WO 90 00567 A (CANCER RES CAMPAIGN TECH) Α 27,29, 25 January 1990 36 - 39, 44-57 see page 1 - page 10 Patent family members are listed in annex. Further documents are listed in the continuation of box C. ΙX Х Special categories of cited documents: or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "T" later document published after the international filing date "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international "X" document of particular relevance; the claimed invention filing date cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such docu-ments, such combination being obvious to a person skilled in the art. "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "&" document member of the same patent family Date of mailing of the international search report Date of the actual completion of the international search 07/06/1999 20 May 1999 Authorized officer Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tei. (+31-70) 340-2040, Tx. 31 651 epo ni, Ceder, 0 Fax: (+31-70) 340-3016



Inte onal Application No PCT/GB 98/03766

ategory *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
(KORNBLIHTT ET AL.: "Human mRNA for fibronectin" EMBL SEQUENCE DATABASE,7 November 1985, XP002103220 HEIDELBERG DE	1-3,6
ζ.	Ac X02761 see the whole document -& KORNBLIHTT ET AL.: "Primary structure of human fibronectin: differential splicing may generate at least 10 polypeptides from a single gene" THE EMBO JOURNAL, vol. 4, no. 7, 1985, pages 1755-1759, XP002051533 see abstract see page 1759, left-hand column	1-3,6, 10-13, 27,29
(KORNBLIHTT ET AL.: "Human fibronectin precursor" SWISSPROT SEQUENCE DATA BASE,21 July 1986, XP002103221 Ac P02751	10-13, 27,29
K	see the whole document & KORNBLIHTT ET AL.: "Primary structure of human fibronectin: differential splicing may generate at least 10 polypeptides from a single gene" THE EMBO JOURNAL, vol. 4, no. 7, 1985, pages 1755-1759, XP002051533 see abstract see page 1759, left-hand column	1-3,6, 10-13, 27,29
X	EP 0 207 751 A (DELTA BIOTECHNOLOGY LTD) 7 January 1987 see abstract; claims; figures 2,3 see page 13, line 30 - page 15, line 10	1,3, 6-10,12, 13,27,29
X	"Homo sapiens fibronectin splice form ED-A" PIR1 SEQUENCE DATA BASE,27 November 1985, XP002103253 Ac FNHU see the whole document & DEAN ET AL.: "Cloning and analysis of the promoter region of the human fibronectin gene" PROC. NATL. ACAD. SCI. U.S.A., vol. 84, 1987, pages 1876-1880,	10-13, 27,29
X	EP 0 344 134 A (IST NAZ RIC SUL CANCRO) 29 November 1989 see abstract; figure 1	19-24, 26,28,30



Inte onal Application No PCT/GB 98/03766

	ation) DOCUMENTS CONSIDERED TO BE RELEVANT	Pologant to alaim Ma
Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Ą	US 5 571 679 A (SEKIGUCHI KIYOTOSHI ET AL) 5 November 1996	14-17, 24,25,27
4	US 5 629 291 A (RUOSLAHTI ERKKI I ET AL) 13 May 1997	10,29, 36,47, 48,53-55
	see abstract see column 1, line 29 - line 40 see column 1, line 55 - line 57	

In...rnational application No.

PCT/GB 98/03766

B x i Observations where certain claims were found unsear hable (C ntinuation of item 1 of first sheet)
This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:
1. X Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely: see FURTHER INFORMATION sheet PCT/ISA/210
Claims Nos.: because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).
B x II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)
This International Searching Authority found multiple inventions in this international application, as follows: See additional sheet PCT/ISA/210
As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. X As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims: it is covered by claims Nos.:
Remark on Protest The additional search fees were accompanied by the applicant's protest. No protest accompanied the payment of additional search fees.

International Application No. PCT/GB 98 /03766

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-18, 25, 27, 29, 31, 33 and 36-58 in totality, and 24, 34 and 35 partly

Polynucleotide and polypeptide of migration stimulating factor and their uses, and an antibody reactive with the polypeptide, but not with fibronectin, and the use of the antibody.

2. Claims: 19-23, 26, 28, 30 and 32 in totality, and 24, 34 and 35 partly

An antibody reactive with fibronectin but not with the polypeptide of invention I, and its use.

3.

International Application No. PCT/GB 98 \(03766 \)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Although claims 47-50, 52, 53, 55 and 58 are directed to methods of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy

Inte

Information on patent family members

onal Application No PCT/GB 98/03766

Patent document cited in search repo		Publication date	ŧ	catent family member(s)	Publication date
WO 9416085	Α	21-07-1994	US	5830700 A	03-11-1998
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POLYPEPTIDES, POLYNUCLEOTIDES AND USES THEREOF

The present invention relates to polypeptides, polynucleotides and uses thereof and in particular to migration stimulating factor (MSF).

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MSF has been described previously in the following papers. Schor et al (1988) J. Cell Sci. 90: 391-399 shows that foetal and cancer patient fibroblasts produce an autocrine migration stimulating factor not made by normal adult cells. Schor et al (1988) J. Cell Sci. 90: 401-407, shows that fibroblasts from cancer patients display a mixture of both foetal and adult Schor et al (1989) In Vitro 25: 737-746 phenotypic characteristics. describes a mechanism of action of the migration stimulating factor (MSF) produced by fetal and cancer patient fibroblasts and its effect on hyaluronic acid synthesis. Grey et al (1989) Proc. Natl. Acad. Sci. (USA) 86: 2438-2442 describes the purification of the migration stimulating factor produced by fetal and cancer patient fibroblasts but no amino acid sequence information is given. It is suggested that MSF has a molecular weight of 70kDa. Schor & Schor (1990) Cancer Investig. 8: 665-667 describes the characterisation of migration stimulating activity (MSF) and gives evidence for its role in cancer pathogenesis. Picardo et al (1991) Lancet 337: 130-133 describes the presence of migration stimulating activity in the serum of breast cancer patients. Ellis et al (1992) J. Cell Sci. 102: 447-456 describes the antagonistic effects of transforming growth factor-\$1 and MSF on fibroblast migration and hyaluronic acid synthesis and discusses the possible implications for wound healing. Picardo et al (1992) Exp. Mol. Path. 57: 8-21, describes the identification of migration stimulating factor in wound fluid. Irwin et al (1994) J. Cell Sci. 107: 1333-1346, describes the inter- and intra-site heterogeneity in the expression of fetal-like phenotypic characteristics by gingival fibroblasts

and discusses the potential significance for wound healing. Schor et al (1994) Int J Cancer. 59: 25-32 describes the phenotypic heterogeneity in breast fibroblasts and discusses functional anomaly in fibroblasts from histologically normal tissue adjacent to carcinoma. Schor et al (1991) In: Cell Motility Factors (ed. I Goldberg) pp. 127-146, Birkhauser Press, Basel, describes the heterogeneity amongst fibroblasts in the production of migration stimulating factor (MSF) and discusses implications for cancer Schor et al (1993) In: Cell behaviour: Adhesion and pathogenesis. Motility. (ed. G. Evans, C. Wigley and R. Warn) Society for Experimental Biology Symposium No. 47, pp. 234-251, describes the potential structural homology of MSF to the gelatin-binding domain of fibronectin its potential mode of action and possible function in health and disease. A small amount of partial amino acid sequence is given, but this sequence is similar to fibronectin and, in fact, is not present in the MSF which has now been cloned and sequenced in the present work (see below). It is suggested that MSF activity isolated from foetal fibroblast conditioned medium consists of three proteins, one with an apparent molecular weight of 119kDa and a double of 43 and 33kDa, and, indeed, it was suggested that MSF could be a proteotytic degradation product of Schor (1995) In: Epithelial Mesenchymal Interactions in fibronectin. Cancer (eg. I Goldberg and E Rosen). pp. 273-296. Birkhauser Press, Basel, describes fibroblast subpopulations as accelerators of tumor progression and the potential role of migration stimulating factor. MSF is also discussed in Schor et al (1994) In: Mammary Tumorigenesis and Malignant Progression, Kluwer Academic Publishers, Dickson, R. and Lippman, M. (eds).

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Thus, MSF is believed to be produced by fibroblasts obtained from a majority of breast cancer patients and is not made by their normal adult

counterparts. It is believed that measuring the levels of MSF, for example, in circulating blood or in serum or in urine, may be useful in identifying patients who have or are susceptible to cancer, or that it may be useful in prognosing the outcome of cancer. MSF producing fibroblasts are present in patients with a number of common epithelial tumours, such as carcinoma of the breast, lung and colon, as well as melanoma, and soft tissue sarcoma.

It is believed that it may be particularly useful to measure the levels of MSF in identifying patients who have or are susceptible to breast cancer, or in prognosing the outcome of breast cancer.

In addition, it is believed that MSF may be useful in wound healing since it is present in a majority of wound fluid samples. The directed migration of fibroblasts into the wound site and the transient increase in hyaluronic acid in granulation tissue during the wound healing response are both consistent with the involvement of MSF. (MSF stimulates the synthesis of a high molecular weight species of hyaluronic acid).

20 MSF is known to be related to fibronectin since certain antibodies raised to MSF also bind to fibronectin.

Fibronectin is a widely distributed glycoprotein present at high concentrations in most extracellular matrices, in plasma (300 µg/ml), and in other body fluids. Fibronectin is a prominent adhesive protein and mediates various aspects of cellular interactions with extracellular matrices including migration. Its principal functions appear to be in cellular migration during development and wound healing, regulation of cell growth and differentiation, and haemostasis/thrombosis.

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Further progress in understanding MSF was hindered by the fact that it has not been clear whether MSF is a degradation or breakdown product of fibronectin, and because MSF appears to be structurally related to fibronectin.

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We have now discovered that MSF is not a breakdown product of fibronectin but that it appears, quite unexpectedly, to be a "mini" splice variant of fibronectin. The amino acid sequence of MSF, disclosed for the first time herein, reveals unexpected regions of dissimilarity with fibronectin. This has led to previously unavailable methods of measuring, identifying and localising MSF becoming available. The availability of a polynucleotide encoding MSF, disclosed for the first time herein, makes available methods for producing MSF and useful variants thereof, and makes available new methods of specifically identifying, measuring and localising MSF.

A first aspect of the invention provides a recombinant polynucleotide encoding a polypeptide comprising the amino acid sequence

NLVATCLPVRASLPHRLN V TGASKSK MLRGPGPGLLL L A QCLGTAVPS Q S Ρ v А V S Q S КP G С Y D N G К RQAQQMVQP F E R T Y L G N A L V C Т С Y G G S R G N M Q Q WТ Y Y 25 F T G N R V G D Т E R P ΕE Т С D ΚY С A G R G R I S Т IANRC Н E G T C I G С D T WRRP ΗE Т G G Y M L Ε C V CLG Ν DHAAGT S YVVGETW ΕK Р C F TCLGEGSGRI Т CTSRNRCN D O D V D C 30 R G Ν L L I C G Ν RIGDTW SKKDN Q С V V Т S S G S G Р F D R A Α V ŘН Т S Q T V V T D S G V Y S V G M QWLK Ρ G Н С V S С Q E TAV Т Q T Y G GNSN G С Т С L G N DRTDS Ρ F Т Y T Т SNY EQDQK Ν 35 V GGNSNGALCHF Ρ F LYNNHN Q T R QNYDADQKFGF NMKWCGT Т С SEGRRD HEEICTTNEGVMY RIGDQWDKQHD М G Н MMR TCVGNGRGEW T С Ι Α Y S LRDQ С Ι V D D Ι Q NDTFHKRHEEGHMLNC TCFG Q G R G R WKC D P V D Q C Q D S E T G T F Y Q I G D S W E K Y V H G 40

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QCYCYGRGIGEWHCQPLQTYPSSSGPVEVF
ITETPSQPNSHPIQWNAPQPSHISKYILRW
RPVSIPPRNLGY
[SEQ ID NO: 1]
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or variants or fragments or fusions or derivatives thereof, or fusions of said variants or fragments or derivatives.

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Figure 2 shows the amino acid sequence encoded by the cDNA insert in pMSF1 α which contains the coding sequence for human migration stimulating factor (MSF). Preferably the amino acid sequence is based on that between the most N-terminal methionine and the most C-terminal stop codon (which are marked X). Thus, it is preferred if the polynucleotide encodes a polypeptide comprising the amino acid sequence shown in Figure 2 labelled pMSF1 α between positions 19 and 660 (ie. starting MLRGPG... as marked and encoding ...LGY as marked), or variants of fragments or fusions or derivatives thereof or fusions of said variants or fragments.

Throughout the specification where the term MSF is used, and the context does not indicate otherwise, it includes a polypeptide which has an amino acid sequence given in Figure 2 labelled pMSF1 α and, in particular, the amino acid sequence given between positions 19 and 660.

Amino acid residues are given in standard single letter code or standard three letter code throughout the specification.

It will be appreciated that the recombinant polynucleotides of the invention are not polynucleotides which encode fibronectin or fragments of fibronectin such as the gelatin binding domain. Preferably, the fragments and variants and derivatives are those that include a polynucleotide which encodes a portion or portions of MSF which are portions that distinguish

MSF from fibronectin and which are described in more detail below and by reference to Figure 2.

The polynucleotide may be DNA or RNA but it is preferred if it is DNA.

The polynucleotide may or may not contain introns. It is preferred that it does not contain introns and it is particularly preferred if the

polynucleotide is a cDNA.

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A polynucleotide of the invention is one which comprises the polynucleotide whose sequence is given in Figure 1. Thus, a polynucleotide of the invention includes the sequence

CAAACTTGGT GGCAACTTGC CTCCCGGTGC GGGCGTCTCT CCCCCACCGT CTCAACATGC TTAGGGGTCC GGGGCCCGGG CTGCTGCTGC TGGCCGTCCA GTGCCTGGGG ACAGCGGTGC CCTCCACGGG AGCCTCGAAG AGCAAGAGGC 15 AGGCTCAGCA AATGGTTCAG CCCCAGTCCC CGGTGGCTGT CAGTCAAAGC AAGCCCGGTT GTTATGACAA TGGAAAACAC TATCAGATAA ATCAACAGTG GGAGCGGACC TACCTAGGCA ATGCGTTGGT TTGTACTTGT TATGGAGGAA GCCGAGGTTT TAACTGCGAG AGTAAACCTG AAGCTGAAGA GACTTGCTTT 20 GACAAGTACA CTGGGAACAC TTACCGAGTG GGTGACACTT ATGAGCGTCC TAAAGACTCC ATGATCTGGG ACTGTACCTG CATCGGGGCT GGGCGAGGGA GAATAAGCTG TACCATCGCA AACCGCTGCC ATGAAGGGGG TCAGTCCTAC AAGATTGGTG ACACCTGGAG GAGACCACAT GAGACTGGTG GTTACATGTT AGAGTGTGTG TGTCTTGGTA ATGGAAAAGG AGAATGGACC TGCAAGCCCA 25 TAGCTGAGAA GTGTTTTGAT CATGCTGCTG GGACTTCCTA TGTGGTCGGA GAAACGTGGG AGAAGCCCTA CCAAGGCTGG ATGATGGTAG ATTGTACTTG CCTGGGAGAA GGCAGCGGAC GCATCACTTG CACTTCTAGA AATAGATGCA ACGATCAGGA CACAAGGACA TCCTATAGAA TTGGAGACAC CTGGAGCAAG AAGGATAATC GAGGAAACCT GCTCCAGTGC ATCTGCACAG GCAACGGCCG AGGAGAGTGG AAGTGTGAGA GGCACACCTC TGTGCAGACC ACATCGAGCG 30 GATCTGGCCC CTTCACCGAT GTTCGTGCAG CTGTTTACCA ACCGCAGCCT CTACTCTGTG GGGATGCAGT GGCTGAAGAC ACAAGGAAAT AAGCAAATGC TTTGCACGTG CCTGGGCAAC GGAGTCAGCT GCCAAGAGAC AGCTGTAACC 35 CAGACTTACG GTGGCAACTC AAATGGAGAG CCATGTGTCT TACCATTCAC CTACAACGAC AGGACGACA GCACAACTTC GAATTATGAG CAGGACCAGA AATACTCTTT CTGCACAGAC CACACTGTTT TGGTTCAGAC TCGAGGAGGA AATTCCAATG GTGCCTTGTG CCACTTCCCC TTCCTATACA ACAACCACAA TTACACTGAT TGCACTTCTG AGGGCAGAAG AGACAACATG AAGTGGTGTG GGACCACACA GAACTATGAT GCCGACCAGA AGTTTGGGTT 40 CTGCCCCATG GCTGCCCACG AGGAAATCTG CACAACCAAT GAAGGGGTCA TGTACCGCAT TGGAGATCAG TGGGATAAGC AGCATGACAT GGGTCACATG ATGAGGTGCA CGTGTGTTGG GAATGGTCGT GGGGAATGGA CATGCATTGC CTACTCGCAG CTTCGAGATC AGTGCATTGT TGATGACATC ACTTACAATG TGAACGACAC

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It is particularly preferred if the polynucleotide of the invention is one which comprises the polynucleotide whose sequence is given between positions 57 and 1982 in Figure 1 since this is believed to be the coding sequence for human MSF.

The invention includes a polynucleotide comprising a fragment of the recombinant polynucleotide of the first aspect of the invention. Preferably, the polynucleotide comprises a fragment which is at least 10 nucleotides in length, more preferably at least 14 nucleotides in length and still more preferably at least 18 nucleotides in length. Such polynucleotides are useful as PCR primers.

A "variation" of the polynucleotide includes one which is (i) usable to produce a protein or a fragment thereof which is in turn usable to prepare antibodies which specifically bind to the protein encoded by the said polynucleotide or (ii) an antisense sequence corresponding to the polynucleotide or to a variation of type (i) as just defined. For example, different codons can be substituted which code for the same amino acid(s) as the original codons. Alternatively, the substitute codons may code for a different amino acid that will not affect the activity or immunogenicity of the protein or which may improve or otherwise modulate its activity or immunogenicity. For example, site-directed mutagenesis or other

techniques can be employed to create single or multiple mutations, such as replacements, insertions, deletions, and transpositions, as described in Botstein and Shortle, "Strategies and Applications of *In Vitro* Mutagenesis," *Science*, 229: 193-210 (1985), which is incorporated herein by reference. Since such modified polynucleotides can be obtained by the application of known techniques to the teachings contained herein, such modified polynucleotides are within the scope of the claimed invention.

Moreover, it will be recognised by those skilled in the art that the polynucleotide sequence (or fragments thereof) of the invention can be used to obtain other polynucleotide sequences that hybridise with it under conditions of high stringency. Such polynucleotides includes any genomic DNA. Accordingly, the polynucleotide of the invention includes polynucleotide that shows at least 55 per cent, preferably 60 per cent, and more preferably at least 70 per cent and most preferably at least 90 per cent homology with the polynucleotide identified in the method of the invention, provided that such homologous polynucleotide encodes a polypeptide which is usable in at least some of the methods described below or is otherwise useful. It is particularly preferred that in this embodiment, the polynucleotide is one which encodes a polypeptide containing a portion or portions that distinguish MSF from fibronectin.

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It is believed that MSF is found in mammals other than human. The present invention therefore includes polynucleotides which encode MSF from other mammalian species including rat, mouse, cow, pig, sheep, rabbit and so on.

Per cent homology can be determined by, for example, the GAP program of the University of Wisconsin Genetic Computer Group.

DNA-DNA, DNA-RNA and RNA-RNA hybridisation may be performed in aqueous solution containing between 0.1XSSC and 6XSSC and at temperatures of between 55°C and 70°C. It is well known in the art that the higher the temperature or the lower the SSC concentration the more stringent the hybridisation conditions. By "high stringency" we mean 2XSSC and 65°C. 1XSSC is 0.15M NaCl/0.015M sodium citrate. Polynucleotides which hybridise at high stringency are included within the scope of the claimed invention.

"Variations" of the polynucleotide also include polynucleotide in which relatively short stretches (for example 20 to 50 nucleotides) have a high degree of homology (at least 80% and preferably at least 90 or 95%) with equivalent stretches of the polynucleotide of the invention even though the overall homology between the two polynucleotides may be much less. This is because important active or binding sites may be shared even when the general architecture of the protein is different.

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By "variants" of the polypeptide we include insertions, deletions and substitutions, either conservative or non-conservative, where such changes do not substantially alter the activity of the said MSF.

Variants and variations of the polynucleotide and polypeptide include natural variants, including allelic variants and naturally-occurring mutant forms.

MSF may be assessed in bioassays based on its stimulation of adult skin fibroblast migration, for example, as is described in Picardo *et al* (1991) *The Lancet* 337, 130-133. Specificity for MSF may be inferred by neutralisation of migration stimulating activity by anti-MSF polyclonal antibodies (as herein disclosed). MSF may also be assayed using immunological techniques such as ELISA and the like.

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By "conservative substitutions" is intended combinations such as Gly, Ala; Val, Ile, Leu; Asp, Glu; Asn, Gln; Ser, Thr; Lys, Arg; and Phe, Tyr.

Such variants may be made using the methods of protein engineering and site-directed mutagenesis well known in the art.

15 Preferably, the variant or variation of the polynucleotide encodes a MSF that has at least 30%, preferably at least 50% and more preferably at least 70% of the activity of a natural MSF, under the same assay conditions.

By "fragment of MSF" we include any fragment which retains activity or which is useful in some other way, for example, for use in raising antibodies or in a binding assay, but which is not a fragment of MSF which could also be a fragment of fibronectin.

By "fusion of MSF" we include said MSF fused to any other polypeptide.

For example, the said protein kinase may be fused to a polypeptide such as glutathione-S-transferase (GST) or protein A in order to facilitate purification of MSF, or it may be fused to some other polypeptide which imparts some desirable characteristics on the MSF fusion. Fusions to any

variant, fragment or derivative of MSF are also included in the scope of the invention.

A further aspect of the invention provides a replicable vector comprising a recombinant polynucleotide encoding MSF, or a variant, fragment, derivative or fusion of MSF or a fusion of said variant, fragment or derivative.

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variety of methods have been developed to operably link DNA, polynucleotides, especially to vectors for example via cohesive termini. complementary complementary For instance, homopolymer tracts can be added to the DNA segment to be inserted to the vector DNA. The vector and DNA segment are then joined by hydrogen bonding between the complementary homopolymeric tails to form recombinant DNA molecules.

Synthetic linkers containing one or more restriction sites provide an alternative method of joining the DNA segment to vectors. The DNA segment, generated by endonuclease restriction digestion as described earlier, is treated with bacteriophage T4 DNA polymerase or *E. coli* DNA polymerase I, enzymes that remove protruding, 3'-single-stranded termini with their 3'-5'-exonucleolytic activities, and fill in recessed 3'-ends with their polymerizing activities.

The combination of these activities therefore generates blunt-ended DNA segments. The blunt-ended segments are then incubated with a large molar excess of linker molecules in the presence of an enzyme that is able to catalyze the ligation of blunt-ended DNA molecules, such as bacteriophage T4 DNA ligase. Thus, the products of the reaction are

DNA segments carrying polymeric linker sequences at their ends. These DNA segments are then cleaved with the appropriate restriction enzyme and ligated to an expression vector that has been cleaved with an enzyme that produces termini compatible with those of the DNA segment.

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Synthetic linkers containing a variety of restriction endonuclease sites are commercially available from a number of sources including International Biotechnologies Inc, New Haven, CN, USA.

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A desirable way to modify the DNA encoding the polypeptide of the invention is to use the polymerase chain reaction as disclosed by Saiki et al (1988) Science 239, 487-491. This method may be used for introducing the DNA into a suitable vector, for example by engineering in suitable restriction sites, or it may be used to modify the DNA in other useful

ways as is known in the art.

In this method the DNA to be enzymatically amplified is flanked by two specific primers which themselves become incorporated into the amplified DNA. The said specific primers may contain restriction endonuclease recognition sites which can be used for cloning into expression vectors using methods known in the art.

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The DNA (or in the case of retroviral vectors, RNA) is then expressed in a suitable host to produce a polypeptide comprising the compound of the invention. Thus, the DNA encoding the polypeptide constituting the compound of the invention may be used in accordance with known techniques, appropriately modified in view of the teachings contained herein, to construct an expression vector, which is then used to transform an appropriate host cell for the expression and production of the

polypeptide of the invention. Such techniques include those disclosed in US Patent Nos. 4,440,859 issued 3 April 1984 to Rutter et al, 4,530,901 issued 23 July 1985 to Weissman, 4,582,800 issued 15 April 1986 to Crowl, 4,677,063 issued 30 June 1987 to Mark et al, 4,678,751 issued 7 July 1987 to Goeddel, 4,704,362 issued 3 November 1987 to Itakura et al, 4,710,463 issued 1 December 1987 to Murray, 4,757,006 issued 12 July 1988 to Toole, Jr. et al, 4,766,075 issued 23 August 1988 to Goeddel et al and 4,810,648 issued 7 March 1989 to Stalker, all of which are incorporated herein by reference.

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The DNA (or in the case of retroviral vectors, RNA) encoding the polypeptide constituting the compound of the invention may be joined to a wide variety of other DNA sequences for introduction into an appropriate host. The companion DNA will depend upon the nature of the host, the manner of the introduction of the DNA into the host, and whether episomal maintenance or integration is desired.

Generally, the DNA is inserted into an expression vector, such as a plasmid, in proper orientation and correct reading frame for expression. If necessary, the DNA may be linked to the appropriate transcriptional and translational regulatory control nucleotide sequences recognised by the desired host, although such controls are generally available in the expression vector. The vector is then introduced into the host through standard techniques. Generally, not all of the hosts will be transformed by the vector. Therefore, it will be necessary to select for transformed host cells. One selection technique involves incorporating into the expression vector a DNA sequence, with any necessary control elements, that codes for a selectable trait in the transformed cell, such as antibiotic resistance.

Alternatively, the gene for such selectable trait can be on another vector, which is used to co-transform the desired host cell.

Host cells that have been transformed by the recombinant DNA of the invention are then cultured for a sufficient time and under appropriate conditions known to those skilled in the art in view of the teachings disclosed herein to permit the expression of the polypeptide, which can then be recovered.

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- Many expression systems are known, including bacteria (for example *E. coli* and *Bacillus subtilis*), yeasts (for example *Saccharomyces cerevisiae*), filamentous fungi (for example *Aspergillus*), plant cells, animal cells and insect cells.
- 15 The vectors typically include a prokaryotic replicon, such as the ColE1 ori, for propagation in a prokaryote, even if the vector is to be used for expression in other, non-prokaryotic, cell types. The vectors can also include an appropriate promoter such as a prokaryotic promoter capable of directing the expression (transcription and translation) of the genes in a bacterial host cell, such as E. coli, transformed therewith.

A promoter is an expression control element formed by a DNA sequence that permits binding of RNA polymerase and transcription to occur. Promoter sequences compatible with exemplary bacterial hosts are typically provided in plasmid vectors containing convenient restriction sites for insertion of a DNA segment of the present invention.

Typical prokaryotic vector plasmids are pUC18, pUC19, pBR322 and pBR329 available from Biorad Laboratories, (Richmond, CA, USA) and pTrc99A and pKK223-3 available from Pharmacia, Piscataway, NI, USA.

- A typical mammalian cell vector plasmid is pSVL available from Pharmacia, Piscataway, NJ, USA. This vector uses the SV40 late promoter to drive expression of cloned genes, the highest level of expression being found in T antigen-producing cells, such as COS-1 cells.
- An example of an inducible mammalian expression vector is pMSG, also available from Pharmacia. This vector uses the glucocorticoid-inducible promoter of the mouse mammary tumour virus long terminal repeat to drive expression of the cloned gene.
- Useful yeast plasmid vectors are pRS403-406 and pRS413-416 and are generally available from Stratagene Cloning Systems, La Jolla, CA 92037, USA. Plasmids pRS403, pRS404, pRS405 and pRS406 are Yeast Integrating plasmids (YIps) and incorporate the yeast selectable markers HIS3, TRP1, LEU2 and URA3. Plasmids pRS413-416 are Yeast Centromere plasmids (Ycps).

Other vectors and expression systems are well known in the art for use with a variety of host cells.

The present invention also relates to a host cell transformed with a polynucleotide vector construct of the present invention. The host cell can be either prokaryotic or eukaryotic. Bacterial cells are preferred prokaryotic host cells and typically are a strain of *E. coli* such as, for example, the *E. coli* strains DH5 available from Bethesda Research

Laboratories Inc., Bethesda, MD, USA, and RR1 available from the American Type Culture Collection (ATCC) of Rockville, MD, USA (No ATCC 31343). Preferred eukaryotic host cells include yeast, insect and mammalian cells, preferably vertebrate cells such as those from a mouse, rat, monkey or human fibroblastic and kidney cell lines. Yeast host cells include YPH499, YPH500 and YPH501 which are generally available from Stratagene Cloning Systems, La Jolla, CA 92037, USA. Preferred mammalian host cells include Chinese hamster ovary (CHO) cells available from the ATCC as CCL61, NIH Swiss mouse embryo cells NIH/3T3 available from the ATCC as CRL 1658, monkey kidney-derived COS-1 cells available from the ATCC as CRL 1650 and 293 cells which are human embryonic kidney cells. Preferred insect cells are Sf9 cells which can be transfected with baculovirus expression vectors.

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Transformation of appropriate cell hosts with a DNA construct of the 15 present invention is accomplished by well known methods that typically depend on the type of vector used. With regard to transformation of prokaryotic host cells, see, for example, Cohen et al (1972) Proc. Natl. Acad. Sci. USA 69, 2110 and Sambrook et al. (1989) Molecular Cloning. A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring 20 Harbor, NY. Transformation of yeast cells is described in Sherman et al (1986) Methods In Yeast Genetics, A Laboratory Manual, Cold Spring Harbor, NY. The method of Beggs (1978) Nature 275, 104-109 is also useful. With regard to vertebrate cells, reagents useful in transfecting 25 such cells, for example calcium phosphate and DEAE-dextran or liposome formulations, are available from Stratagene Cloning Systems, or Life Technologies Inc., Gaithersburg, MD 20877, USA.

Electroporation is also useful for transforming and/or transfecting cells and is well known in the art for transforming yeast cell, bacterial cells, insect cells and vertebrate cells.

For example, many bacterial species may be transformed by the methods described in Luchansky et al (1988) Mol. Microbiol. 2, 637-646 incorporated herein by reference. The greatest number of transformants is consistently recovered following electroporation of the DNA-cell mixture suspended in 2.5X PEB using 6250V per cm at 25µFD.

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Methods for transformation of yeast by electroporation are disclosed in Becker & Guarente (1990) Methods Enzymol. 194, 182.

Successfully transformed cells, ie cells that contain a DNA construct of the present invention, can be identified by well known techniques. For example, cells resulting from the introduction of an expression construct of the present invention can be grown to produce the polypeptide of the invention. Cells can be harvested and lysed and their DNA content examined for the presence of the DNA using a method such as that described by Southern (1975) *J. Mol. Biol.* 98, 503 or Berent *et al* (1985) *Biotech.* 3, 208. Alternatively, the presence of the protein in the supernatant can be detected using antibodies as described below.

In addition to directly assaying for the presence of recombinant DNA,
successful transformation can be confirmed by well known immunological
methods when the recombinant DNA is capable of directing the expression
of the protein. For example, cells successfully transformed with an
expression vector produce proteins displaying appropriate antigenicity.

Samples of cells suspected of being transformed are harvested and assayed for the protein using suitable antibodies.

Thus, in addition to the transformed host cells themselves, the present invention also contemplates a culture of those cells, preferably a monoclonal (clonally homogeneous) culture, or a culture derived from a monoclonal culture, in a nutrient medium.

A further aspect of the invention provides a method of making MSF or a variant, derivative, fragment or fusion thereof or a fusion of a variant, fragment or derivative, the method comprising culturing a host cell comprising a recombinant polynucleotide or a replicable vector which encodes said MSF or variant or fragment or derivative or fusion, and isolating said MSF or a variant, derivative, fragment or fusion thereof of a fusion or a variant, fragment or derivative from said host cell.

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Methods of cultivating host cells and isolating recombinant proteins are well known in the art. It will be appreciated that, depending on the host cell, the MSF produced may differ from that which can be isolated from nature. For example, certain host cells, such as yeast or bacterial cells, either do not have, or have different, post-translational modification systems which may result in the production of forms of MSF which may be post-translationally modified in a different why to MSF isolated from nature. It is preferred if the host cell is a non-human host cell; move preferably it is not a mammalian cell.

It is preferred that recombinant MSF is produced in a eukaryotic system, such as an insect cell.

A further aspect of the invention provides MSF or a variant, fragment, derivative or fusion thereof or a fusion of a variant, fragment or derivative obtainable by the methods herein disclosed.

A further aspect of the invention provides a polypeptide comprising the amino acid sequence

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NLVATCLPVRASLPHRLN
         G P
            G P
                G
                 L
                   LLL
                         Α
                           V
                            Q
                              C
                                LGTAVPSTGASKSK
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                                  CY
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     PPPYGHCVTDSG
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                       G A
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                                  P
                                    F
                                       Y
       V
         Q
           Т
            RGGNSN
                              H F
                                     L
                                         N
                                           N
                                             Н
          RRDNMKWCGT
    HEEI
25
         VGNGRGEWT
                         С
                           1
                              Y
                            A
                                S
                                  Q L R D
                                         Q
                                           С
                                             I
                                  С
         D
            F
              HKRHEE
                         G
                           H
                            М
                              LN
                                    T
                                      С
                                       F
                                         G
       V D
              QDSETGTF
                            Y
                              QIGDSWEK
                                             YV
            С
                                                Н
                                                  G
       Y C
           YGRGIGEWHCQPLQTYPSS
                                            SGP
                                                  V
    I T E T P S Q P N S H P I Q W N A P Q P S H I S K Y I L R W
30
    RPVSIPPRNLGY
```

[SEQ ID NO: 1]

or variants or fragments or fusions or derivatives thereof or fusions of said variants or fragments or derivatives.

35 Thus, a polypeptide of the invention includes

```
NLVATCLPVRASLPHRLN
     LRGPGPGLLL
                        L A
                            V
                              0
                                С
                                      TAVPSTGASKSK
               ٧
                    Q S
                        P
                          V
                            Α
                              ν
                                S
                                  Q
                                       ΡG
                                           C
                                             Υ
                                               D
                                                   G
           QM
                 Q
                  ₽
                                    S
                                      K
40
    I N
       Q
           WE
               R
                T
                  Y
                    L
                      G
                        N A
                            L
                              v
                                С
                                  Т
                                    С
                                      Y
                                         G
         Q
         E E
               C F
                  D K
                      Y
                        T
                          GNT
                                  R
                                    V
                                      GDT
             T
       CTCIGAGRGRISCT
                                  I
                                    ANRC
                                           ΗE
                                    V
       T W
           RRPHETGG
                          Y
                            M
                              L
                                £
                                  C
                                      С
                                        L
                                           Ν
                                         G
                              Y
       Α
         E
           K
             С
               F
                 DHAA
                        G
                          T
                            s
                                ٧
                                  V
                                    G
                                      E
                                        T
                                           E
                                             K
                                               ₽
45
           C L
                            Т
                              С
                                \mathtt{T} \ \mathtt{S} \ \mathtt{R} \ \mathtt{N} \ \mathtt{R} \ \mathtt{C} \ \mathtt{N}
      DCT
               GEGSGRI
                                             D
                                               Q
                                                 D
     IGDTWSKKDNRGNLL
                                  Q C
                                      I C
                                         TGNGRG
     RHTSVQTTSSGSGPFTDVRAAVYQPQPHP
    Q P P P Y G H C V T D S G V V Y S V G M Q W L K T Q G N K Q
```

```
TQTYGGNSNG
   MLCTCLGNGVSCQE
                          TAV
                   D S
                      T
                        Т
                          S
                           NYE
                                Q
                                  D
                                    QK
                                       Y
                                        S
                  T
           Y
             N
              D R
              GNS
                   NGA
                        L
                          С
                               Ρ
           R G
                           NYDA
                          Q
                                  DQK
                                       F
                                        GF
   SEGRRDNMKW
                   С
                     G
                      Т
                        Т
                     M Y
                        R I
                           G
                             D
                               Q
                                W
                                  D
                                   K
                                     QH
     ΕE
        I C
           TTNE
                 G
                   ν
                   W
                     T
                       С
                        1
                          Α
                           Y
                             S
                                L
                                  R
                                   D
      С
        VGNG
              R G
                  E
                               С
                                Т
                                  С
                                    F
                                     G
                                       0
      N
        D T
           F
             Н
              K
                R
                 Н
                   E
                     Ε
                      G
                        н
                          MLN
                                        G R
                      Т
                        F
                          Y
                           QI
                               G
                                D S
                                   W
                S
                 E
                   T
                     G
      v
        D
           С
             Q
              D
            R
              G
                IGE
                     WHCQPLQ
                                Т
                                  Y
                                   P S
                                       SSGP
      Y
         Y G
           SQPNSHPIQWNAPQPSHISKYIL
10
         IPPRNLGY
```

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[SEQ ID NO: 1]

Preferably, the polypeptide comprises the amino acid sequence shown in Figure 2 labelled pMSF1 α between positions 19 and 660, or variants or fragments or fusions or derivatives thereof or fusions of said variants or fragments or derivatives.

It will be appreciated that the polypeptides of the invention are not fibronectin or fragments of fibronectin such as the gelatin binding domain. Preferably, the fragments and variants and derivatives are those that include a portion or portions of MSF which are portions that distinguish MSF from fibronectin and which are described in more detail below and by reference to Figure 2.

25 Preferably, the polypeptide of the invention is one which has migration stimulating factor activity.

Further aspects of the invention provide antibodies which are selective for MSF (and do not cross react with fibronectin) and antibodies which are selective for fibronectin (and do not cross react with MSF).

By "selective" we include antibodies which bind at least 10-fold more strongly to one polypeptide than to the other (ie MSF vs fibronectin); preferably at least 50-fold more strongly and more preferably at least 100-fold more strongly.

Such antibodies may be made by methods well known in the art using the information concerning the differences in amino acid sequence between MSF and fibronectin disclosed herein. In particular, the antibodies may be polyclonal or monoclonal.

Suitable monoclonal antibodies which are reactive as said may be prepared by known techniques, for example those disclosed in "Monoclonal Antibodies: A manual of techniques", H Zola (CRC Press, 1988) and in "Monoclonal Hybridoma Antibodies: Techniques and Applications", SGR Hurrell (CRC Press, 1982). Polyclonal antibodies may be produced which are polyspecific or monospecific. It is preferred that they are monospecific.

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One embodiment provides an antibody reactive towards the polypeptide whose amino acid sequence is

```
NLVATCLPVRASLPHRLN
   MLRGPGPGLLLLAV
                          QCLGTAVPSTGASKSK
20
                       V
                        ΑV
   RQAQQMV
              QPQSP
                            SQSKPGC
                                       Y D
                                          N
           E R T
                Y
                  L
                   G
                     ΝA
                        L
                          V
                            С
                             T
                               С
                                 Y
                                  G
                                    G
                                     S
              F
                D K Y
                       G N T
                            Y
                             R
                               V
                                      Y
      Α
        ΕE
             С
                     Т
           T
                                 G D
                                    T
                                       E R
                                          P
                                            K
          CIGAGRGRIS
                          С
                             IANRCHE
                                        GG
   GDTWRRPHET
                   GGYMLECV
                                 CLGNGK
25
     IAEK
           CFDHAAGTSY
                            V
                             V
                               GETW
                                     EKP
                                          Y
                                            QG
      С
             GE
                G
                  s
                    G
                     R
                       I
                         Т
                          С
                            Т
                             s
                               R
                                 N
                                  R
                                    С
                                     N
      G
        D T
             SKKDNRGNL
                                            GE
           W
                            L
                             Q
                               C
                                 I C
                                    T
                                     GNGR
        T S V
                TSSGSGPF
                             TDVRAAVY
   ERH
             QT
                                          Q P
         YGHCVTDSGVVYSVGM
      P P
                                    QWLKT
    L C T C L G N G V S C Q E T A V T Q T Y G G N S N L P F T Y N D R T D S T T S N Y E Q D Q K Y S F C
30
      v
          Т
           R
             G
               G
                N
                  S
                    N
                     G
                       Α
                         L
                          С
                            H F
                               P
                                 F
                                  L
                                    Y
                                      N N
                                         Н
                  W
                    С
                       Т
                         Т
                          QN
                               D
      G
        R
         RDN
              M K
                     G
                             Y
                                 Α
                                  D
                                            С
                                    Q
                                     ĸ
                                       F
                                         G
                                          F
      Ε
        I C
           Т
             T N E
                  G
                    V M
                       Y
                         R
                          I
                            GDQWDK
                                      ОН
                                         DMG
35
      С
             GRGEWT
                        IAYSQLRD
        VGN
                       С
                                     Q C
                                        ΙVD
      NDTFHKRHEEGHMLNCTCF
                                     G Q
                                        GRG
      ٧
          Q C
             Q D
                S E
                    Т
                     G
                       Т
                         F
                          ΥQ
                             IGDS
                                    W
                                     ΕK
                                            H G
     С
      Y C
                ΙG
                   EWHCQPLQT
                                  Y
                                    Ρ
                                      S
          Y
           G
             R G
                                       S
                                         S G
                                            P
           SQPNSHPIQWNAPQPSHISKYIL
   ITET
          Ρ
40
   RPVSIPPRNLGY
```

[SEQ ID NO: 1]

or natural variants thereof but not reactive towards fibronectin.

A further embodiment provides an antibody reactive towards the polypeptide whose amino acid sequence is shown in Figure 2 labelled pMSF1 α between positions 19 and 660 or natural variants thereof but not reactive towards fibronectin.

A further embodiment provides an antibody reactive towards an epitope present in the polypeptide whose amino acid sequence is shown in Figure 2 labelled pMSF1 α or natural variants thereof but which epitope is not present in fibronectin.

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A further embodiment provides an antibody reactive towards an epitope present in the polypeptide whose amino acid sequence is

NLVATCLPVRASLPHRLN MLRGPGPGLLLLAVO CLGTAVPSTGASKSK QQMVQPQSPVAV SQSKPGC YDNG QQWERTYLGNALVC Т С Y G G S R G 20 FDKY Т G Ν T Y R V G E Т С C T С I G AGRGRIS Т Ι ΑN R C Н E GWRRPHETGGYMLEC V С LGNGKG IAEKCFDHAAGTSYV VGETW ΕKΡ DCTCLGEGSGRITCTSRNRCNDQDT 25 IGDTWSKKDNRGN L L Q С Ι С T G S \$ G Ρ E D V Н Т S V Q Т T S G Т R Α Α Y Ρ P Ρ Y С VTDS G V V Y S V GMQWΤ. Т G H GNGV S C QΕ T A V T Q G L PFTYNDRTDSTT SNYE D Q K Y S Q 30 A L V QTRGGNS N G С H F P F L N N н G NM K W С G T Т Q Ν Y Đ Α D Q К F G F TNEGVMYRIGDQWDKQH Ε Ι T D G С VGNGRGEWTCIAYSQLRD С Q С V N D T F H K R H E E G H M L N C T C F G QGRG 35 V D Q C Q D S E T G T F Y Q I G D S W E K V Ρ Y Н G GE WHC QP L Q T Y Ρ S s S G P V С Y G R G QP NSHPIQWNAP S H Ι S Y Ε T Р S QP K VSIPPRNLGY

[SEQ ID NO: 1]

between positions 19 and 660 or natural variants thereof but which is epitope is not present in fibronectin.

It is particularly preferred if the antibody is reactive towards a molecule comprising any one of the peptides:

ISKYILRWRPVSIPPRNLGY; [SEQ ID NO: 3]; or QQWERTYLGNALVCTCYGGSR; [SEQ ID NO: 4]; or PCVLPFTYNDRTDSTTSNYEQDQ; [SEQ ID NO: 5]; or TDHTVLVQTRGGNSNGALCH: [SEQ ID NO: 6]; or VGNGRGEWTCIAYSQLRDQCI [SEQ ID NO: 7]

which are found in MSF. The underlined amino acid(s) indicate the difference between MSF and fibronectin.

These peptides contain and flank regions of difference in amino acid sequence between MSF and fibronectin as shown in Figure 2 which are believed to be useful in distinguishing MSF and fibronectin using antibodies.

A further embodiment provides an antibody reactive towards fibronectin but not reactive towards the polypeptide whose amino acid sequence is shown in Figure 2 labelled pMSF1 or natural variants thereof.

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A further embodiment provides an antibody reactive towards fibronectin but not reactive towards the polypeptide whose amino acid sequence is shown in Figure 2 labelled pMSF1 between positions 19 and 660 or natural variants thereof.

25

A further embodiment provides an antibody reactive towards an epitope present in fibronectin but not present in the polypeptide whose amino acid sequence is

PCT/GB98/03766

WO 99/31233

NLVATCLPVRASLPHRLN AVQCLGTAVPSTGASKSK L G GPGLL L YDN QSKPGC QMVQPQSP VAVS Q INQQWERTYLGNALVCTCYGG GNTY R V G D EETCFDKYT RCHE S С T IAN IGAGRGR CTC Ε С ν С L GN RRPHE Т GG Y M L T S ΥV VGET HAAG F D EGSGRI CTSRNRCNDQDT Т С LG ICTGNGR IGDTWSKKDNRGNLL Q С 10 SVQTTSSGSGPF Т D R A Т PYGHC V T D S G V Y S V G M W L Y v QT GGN QE T С T A CTCLGN G v S D S T T SNYE Q D YNDRT RGGNSNGALCHFPFLŸNNH 15 Т QNYDAD QKF RRDNMKWCGTT TTNEGVMYRIGDQ D Q QLRD Q С I CVGNGRGEWT С Α S Ι HMLNCTC NDTFHKRHEEG F G QG R G YQIGDSWEKYVH QDSETGTF 20 GRGIGEWHCQPLQTYPSSSGPVE Y C Y I T E T P S Q P N S H P I Q W N A P Q P S H I S K Y I L R P V S I P P R N L G Y

[SEQ ID NO: 1]

25 or natural variants thereof.

30

A further embodiment provides an antibody reactive towards an epitope present in fibronectin but not present in the polypeptide whose amino acid sequence is shown in Figure 2 labelled pMSF1 α between positions 19 and 660 or natural variants thereof.

It is particularly preferred if the antibody is reactive towards a molecule comprising any one of the peptides:

QQWERTYLGNVLVCTCYGGSR [SEQ ID NO: 8] or

EPCVLPFTYNGRTFYSCTTEGRQDGHLWCSTTSNYEQDQ [SEQ ID NO: 9] or

CTDHTVLVQTQGGNSNGALCH [SEQ ID NO: 10] or

VGNGRGEWTCYAYSQLRDQCI [SEQ ID NO: 11] or

ISKYILRWRPKNSVGRWKEA [SEQ ID NO: 12] or

peptides derived from position 648 onwards in fibronectin as shown in Figure 2. The underlined amino acid(s) indicate the difference between fibronectin and MSF.

These peptides themselves may be useful for raising antibodies, but selective antibodies may be made using smaller fragments of these peptides which contain the region of difference between MSF and fibronectin.

Peptides in which one or more of the amino acid residues are chemically modified, before or after the peptide is synthesised, may be used providing that the function of the peptide, namely the production of specific antibodies in vivo, remains substantially unchanged. modifications include forming salts with acids or bases, especially physiologically acceptable organic or inorganic acids and bases, forming an ester or amide of a terminal carboxyl group, and attaching amino acid protecting groups such as N-t-butoxycarbonyl. Such modifications may protect the peptide from in vivo metabolism. The peptides may be present as single copies or as multiples, for example tandem repeats. tandem or multiple repeats may be sufficiently antigenic themselves to obviate the use of a carrier. It may be advantageous for the peptide to be formed as a loop, with the N-terminal and C-terminal ends joined together, or to add one or more Cys residues to an end to increase antigenicity and/or to allow disulphide bonds to be formed. If the peptide is covalently linked to a carrier, preferably a polypeptide, then the arrangement is preferably such that the peptide of the invention forms a loop.

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According to current immunological theories, a carrier function should be present in any immunogenic formulation in order to stimulate, or enhance stimulation of, the immune system. It is thought that the best carriers embody (or, together with the antigen, create) a T-cell epitope. The

peptides may be associated, for example by cross-linking, with a separate carrier, such as serum albumins, myoglobins, bacterial toxoids and keyhole limpet haemocyanin. More recently developed carriers which induce T-cell help in the immune response include the hepatitis-B core antigen (also called the nucleocapsid protein), presumed T-cell epitopes such as Thr-Ala-Ser-Gly-Val-Ala-Glu-Thr-Thr-Asn-Cys [SEQ ID NO: 13], betagalactosidase and the 163-171 peptide of interleukin-1. The latter compound may variously be regarded as a carrier or as an adjuvant or as both. Alternatively, several copies of the same or different peptides of the invention may be cross-linked to one another; in this situation there is no separate carrier as such, but a carrier function may be provided by such cross-linking. Suitable cross-linking agents include those listed as such in Pierce catalogues, for example glutaraldehyde, Sigma and carbodiimide and succinimidyl 4-(N-maleimidomethyl)cyclohexane-1carboxylate, the latter agent exploiting the -SH group on the C-terminal cysteine residue (if present).

If the peptide is prepared by expression of a suitable nucleotide sequence in a suitable host, then it may be advantageous to express the peptide as a fusion product with a peptide sequence which acts as a carrier. Kabigen's "Ecosec" system is an example of such an arrangement.

The peptide of the invention may be linked to other antigens to provide a dual effect.

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A further aspect of the invention provides a method of making an antibody which is reactive towards the polypeptide whose amino acid sequence is

N L V A T C L P V R A S L P H R L N

30 M L R G P G P G L L L L A V Q C L G T A V P S T G A S K S K

RQAQQMVQPQSPVAVSQSKPGCYDNGKHYQ v С С Y G G S R F N G N Α L Т WE R Т Υ L Т Y R GDT EET С F D K $Y \cdot T$ GN CTIANRCHEG GRGRIS CI G A v CLGN GK G 5 WRRPHET GGYMLEC Y ν G E Е FDHAAGTS V IAEKC Т D C Т CL G E G S G R I Т C S R N R C N D D L Q Ι CTGNGRG W S K K D N R GΝ L С s v S G P F T DVRAAV Y Q s G S T T T Н 10 P YGHC ٧ Т D S G V V Y S VGMQWLKT P G N S S CQETAVT QTYG N LGNGV T C FTYNDRTDSTT SNYE Q DQK Y S F P QTRGGNSNG Α L С Н F P F L Y N N Н N V Т Y D Α F T Q N D Q K G F G R D N M K W С G R RI V Y GDQWDK QHDMG 15 E E T N E G М Ι С T RGEW Т С ΙΑ YSQLRD QCIVD С v GNG V N D T F H K R H E E G H M L N C T C F G Q R G G GTFYQIGDSW DSET Ε К G V D QCQ R GEWHCQP LQT Y P S S S G v С Y С Y G G I IQWNAPQPSHISKYIL P 20 T P S Q P Ν S H v Y ΙP L G S P R N

[SEQ ID NO: 1]

or a natural variant thereof and which is not reactive with fibronectin, the method comprising the steps of, where appropriate, immunising an animal with a peptide which distinguishes MSF from fibronectin and selecting an antibody which binds MSF but does not substantially bind fibronectin. Suitable peptides are disclosed above.

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A still further aspect of the invention provides a method of making an antibody which is reactive towards fibronectin and which is not reactive towards the polypeptide whose amino acid sequence is [SEQ ID NO: 1]

NLVATCLPVRASLPHRLN G P L A v 0 CLGTAVPSTGASKSK G P G L L L QSPVAV QMV SQSKPGCYDN 35 Q 0 Ρ YLGNALV YGGSRG N WERT СТ С F Q $\texttt{C} \ \texttt{F} \ \texttt{D} \ \texttt{K} \ \texttt{Y} \ \texttt{T} \ \texttt{G} \ \texttt{N} \ \texttt{T} \ \texttt{Y} \ \texttt{R} \ \texttt{V}$ GDTY Ε R P K E T R I G Y S С T ANRC Н Ε G G ΙG A G R G Ι T С Ε С ν ĸ R R Ρ Н Ε T G Μ L С L G Ν G 40 Α G Т S Y V V GE T W E P С F Α Ε D Н Ι K D Т С L G E G S GRI Т CTSRNRCND Q GNLL G R W S K K D N RT G N С Ι C D Т Q s v Т T S S G S G P F T D V R A A V Y E Т Н Q T V ν Y S С V D S G v V G М W L K Q Ρ Ρ Y G Н s С E Т Α v T S 45 С T С G Q Q T Y GG N N L G N ΥE QDQKYSF DRTDS Т Т S N P F T Y N CHFP NNHNY V O TRG GNSNGAL FLY QNYDADQKF T T G F С GRRDNMKW C G E I C T T N E.G V М Y R I G DQWDКQН D Μ G Н IAYSQLRDQC 50 GEWT С I V D C V G N G R F H K R H E E G H M L N C T C F G Q G R G R W K C DT

```
D P V D Q C Q D S E T G T F Y Q I G D S W E K Y V H G V R Y Q C Y C Y G R G I G E W H C Q P L Q T Y P S S S G P V E V F I T E T P S Q P N S H P I Q W N A P Q P S H I S K Y I L R W R P V S I P P R N L G Y
```

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or a natural variant thereof, the method comprising the steps of, where appropriate, immunising an animal with a peptide which distinguishes fibronectin from MSF and selecting an antibody which binds fibronectin but does not substantially bind MSF. Suitable peptides are disclosed above.

It will be appreciated that, with the advancements in antibody technology, it may not be necessary to immunise an animal in order to produce an antibody. Synthetic systems, such as phage display libraries, may be used. The use of such systems is included in the methods of the invention.

Before the present invention it was not possible to make use of the differences in amino acid sequence between fibronectin and MSF in order to make antibodies which are useful in distinguishing MSF and fibronectin since it was not known that MSF and fibronectin had significant differences in structure or what those differences were. As is discussed in more detail below, such antibodies are useful in cancer diagnosis. It will also be appreciated that such antibodies which distinguish MSF and fibronectin are also useful research reagents. Suitably, the antibodies of the invention are detectably labelled, for example they may be labelled in such a way that they may be directly or indirectly detected. Conveniently, the antibodies are labelled with a radioactive moiety or a coloured moiety or a fluorescent moiety, or they may be linked to an enzyme. Typically, the enzyme is one which can convert a non-coloured (or non-fluorescent) substrate to a coloured (or fluorescent) product. The antibody may be labelled by biotin (or streptavidin) and then detected indirectly using

streptavidin (or biotin) which has been labelled with a radioactive moiety or a coloured moiety or a fluorescent moiety, or the like or they may be linked to an enzyme of the type described above.

It is particularly preferred if peptides are made, based on the amino acid sequence of MSF and fibronectin, which allow for specific antibodies to be made.

Thus, a further aspect of the invention provides a molecule which is
capable of, following immunisation of an animal if appropriate, giving rise
to antibodies which are reactive towards the polypeptide whose sequence
is

```
NLVATCLPVRASLPHRLN
   MLRGPGPGLLLLAVQCLGTAVPSTGASKSK
15
                PQSPV
                        A V
                            SQSKPG
                                      С
                                       Y
                                         D
                                           N
                                             G K
          QMVQ
      QQWERT
                YLGNA
                        L
                          V
                            С
                              Т
                               С
                                 Y
                                   GG
                                      S
                              R V
                            Y
      AEE
           T C
              F
                D
                  ΚY
                     T
                       G N T
                                 GDT
                                      Y
                                        Ε
                                         R
                                           P
                GRGRISCT
                                    CHE
           I G A
                              IANR
          С
        T
20
                  T G G
                       YMLE
                              С
                               V
                                 С
                                    G N
        WRRPHE
           C F D H A A G T S Y \overline{V}
                              v
                               GET
                                    W
                                      ΕK
        TCLGEGSGRI
                         T
                          С
                            T
                              S R
                                 N R
                                    С
                                      N
                       G
                        Ν
                          L
                            L
                              Q
                                 Ι
                                   С
                                        N
           W S
              K
                KDNR
                               С
                                      G
                  S S G
                       SG
                          Ρ
                            F
                              T
                               D
                                 V R A A
                                         Y
          s v
             Q
               T
                T
                                 G M Q W L K
Q T Y G G N
25
          YGHC
                VTDSGV
                          VY
                             s v
        Ρ
     L C T C L G N G V S C Q E T A V T L P F T Y N D R T D S T T S N Y E
                                           S N
                                 Q
                                   DQKY
                                         S
                                           F
                     G
                       A
                         L
                          С
                            Н
                              F
                               Ρ
                                 F
                                    Y
        QTRGGNS
                    N
      V
                              Y
                    С
                     G
                       T
                         T
                          Q
                            N
                               D
                                 Α
                                   D
                                    Q
                                      К
                                        F
                                         G
                                           F
       GRRDNMKW
           TTNEGVM
                       Y R I
                              D
                                    K
30
                            G
                               Q
                                 WD
                                      QH
                                         D
      Ε
        Ι
          С
        VGNGRGEWT
                         IAYSQLR
                       С
                                    D
           FHKRHEEGHMLNC
                                 T C
                                    F
                                        QG
                                           R
     VNDT
                                      G
      VDQCQDSETG
                       TF
                          YQIG
                                 D S
                                    W
                                      Ε
                                        K
             CYGRGIGEWHC
                                           G
                                             Ρ
      Y
35
                                           KYIL
        Т
          Р
           s
      v
        SIPPRNLGY
                                                [SEQ ID NO: 1]
```

or natural variants thereof but not reactive towards fibronectin.

A still further aspect of the invention provides a molecule which is capable of, following immunisation of an animal if appropriate, giving rise to

antibodies which are reactive towards fibronectin but not reactive towards the polypeptide whose sequence is

```
NLVATCLPVRASLPHRLN
   MLRGPGPG
                  LLL
                       L A V
                             Q C
                                L G
                                    TAV
                                         P S
                         VAVS
                                            Υ
              V Q
                  PQSP
                                Q S
                                    K
                                      Ρ
                                        G
                                          С
                                             D
                                               N
                                                 G
          Q M
                     GNALVC
                                T C
                                    Y
                  Y L
                                      G
                                         S
                                           R
          WERT
         Q
                FDKYT
                         GNTYRV
         Ε
          ΕT
              C
                                    G D
            IGAGRGRISC
                              TIANRCHE
         W
          RRPHETGGYMLE
                                С
                                  V
                                    С
                                      LGN
                                           G
                                             KG
10
                DHAA
                       G
                         T
                           S
                             Y
                               V
                                 V
                                   G
                                    E
                                        W
                                            K
       Α
         EKC
              F
                                          E
                           T
                             С
                               T
                                 S
                                        C N
         Т
              G
                E
                  G
                    S
                     G R
                         I
                                   R
                                    N
                                      R
                                            D
                                               D
                KKDNRGNL
                               L
                                 Q
                                   С
                                    I C
                                        T
                                          G N
                                             G
       G
         D
           Т
            W S
       Н
         Т
           S
            V
              Q
                T
                  Т
                    SSGSGP
                               F
                                 T
                                   D
                                    VRAA
       Ъ
         Ρ
          YGH
                CVTDSGV
                             V
                               Y S
                                  VGMQWLKT
15
                      s c
                             \mathbf{T}
                                 V
       С
                NG
                   V
                         Q
                           Ε
                              Α
                                   Т
                                           G
                                             N
                                               S
         T C
            L
              G
                                    Q
                                      TY
                                          G
       P
         F
           T
            Y
              N
                D
                  R
                    Т
                      D
                       S
                         Т
                           Т
                             s
                               N
                                 Y
                                   E
                                    Q
                                      D
                                        Q
                                          K
                                              S
                       G
                         Α
                           L
                             С
                                   P
                                        Y
       V
           Т
            R
              G
                G
                  N
                    S
                      N
                               Н
                                 F
                                    F
                                      L
                                          N
                                            N
                                              Η
                                               N
              N
                    W
                      CG
                         Т
                           Т
                             Q
                                 Y
                                   D
                                            F
                                               F
       G
          R D
                  K
                               N
                                    Α
                                      Đ
                                        Q
         R
                М
                                          К
              Т
                NE
                    G
                      V M
                         Y
                           R
                             I
                               G
                                D
                                      D
                                        K
         I
           С
            Т
                                   Q
                                          Q
20
                RGEWT
                             Α
       С
           G N G
                         С
                           Ι
                               Y
                                 S
                                    L R
                                        D
                                            С
                                              I
         V
                                   Q
                                          Q
                             М
     V
       NDTFHKRHEEG
                           Н
                               L
                                 N C
                                    Т
                                      С
                                        F
                                          G
                                           Q
                                              G
                                               R
       V
                D
                  S
                    E
                      T
                       G
                         T
                           F
                             Y
                               0
                                 I
                                   G
                                    D
                                      S
                                        W
                                          E
                                            K
                                               V
           0
            C
              Q
                      E W H C
                                      Y P S
                  I
                    G
                             Q P
                                L
                                   Q
                                    T
                                            S
                                               G
       Y
         С
           Y
            G
              R G
                PNSHPIQWNAP
                                    QPSHI
     T E
         T P
            S
              Q
25
```

[SEQ ID NO: 1]

or natural variants thereof.

The molecule is preferably a peptide but may be any molecule which gives rise to the desired antibodies. The molecule, preferably a peptide, is conveniently formulated into an immunological composition using methods well known in the art.

The peptides disclosed above form part of these aspects of the invention.

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Peptides may be synthesised by the Fmoc-polyamide mode of solid-phase peptide synthesis as disclosed by Lu et al (1981) J. Org. Chem. 46, 3433 and references therein. Temporary N-amino group protection is afforded by the 9-fluorenylmethyloxycarbonyl (Fmoc) group. Repetitive cleavage of this highly base-labile protecting group is effected using 20% piperidine in N,N-dimethylformamide. Side-chain functionalities may be protected as their butyl ethers (in the case of serine threonine and tyrosine), butyl

esters (in the case of glutamic acid and aspartic acid), butyloxycarbonyl derivative (in the case of lysine and histidine), trityl derivative (in the case of cysteine) and 4-methoxy-2,3,6-trimethylbenzenesulphonyl derivative (in the case of arginine). Where glutamine or asparagine are C-terminal residues, use is made of the 4,4'-dimethoxybenzhydryl group for protection of the side chain amido functionalities. The solid-phase support is based on a polydimethyl-acrylamide polymer constituted from the three monomers dimethylacrylamide (backbone-monomer), bisacryloylethylene diamine (cross linker) and acryloylsarcosine methyl ester (functionalising agent). The peptide-to-resin cleavable linked agent used is the acid-labile 4-hydroxymethyl-phenoxyacetic acid derivative. All amino derivatives are added as their preformed symmetrical anhydride derivatives with the exception of asparagine and glutamine, which are reversed N, N-dicyclohexyl-carbodiimide/1added using hydroxybenzotriazole mediated coupling procedure. All coupling and deprotection reactions are monitored using ninhydrin, trinitrobenzene sulphonic acid or isotin test procedures. Upon completion of synthesis, peptides are cleaved from the resin support with concomitant removal of side-chain protecting groups by treatment with 95% trifluoroacetic acid containing a 50% scavenger mix. Scavengers commonly used are ethanedithiol, phenol, anisole and water, the exact choice depending on acids of the peptide being synthesised. the constituent amino Trifluoroacetic acid is removed by evaporation in vacuo, with subsequent trituration with diethyl ether affording the crude peptide. Any scavengers present are removed by a simple extraction procedure which on lyophilisation of the aqueous phase affords the crude peptide free of scavengers. Reagents for peptide synthesis are generally available from Calbiochem-Novabiochem (UK) Ltd, Nottingham NG7 Purification may be effected by any one, or a combination of, techniques

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such as size exclusion chromatography, ion-exchange chromatography and (principally) reverse-phase high performance liquid chromatography. Analysis of peptides may be carried out using thin layer chromatography, reverse-phase high performance liquid chromatography, amino-acid analysis after acid hydrolysis and by fast atom bombardment (FAB) mass spectrometric analysis.

It is now possible to make polynucleotides which can distinguish MSF and fibronectin and such polynucleotides are believed to be useful in the diagnosis and prognosis of cancer.

A further aspect of the invention provides a polynucleotide which is capable of distinguishing a polynucleotide which encodes the polypeptide whose sequence is

```
NLVATCLPVRASLPHRLN
                           QCLGTAVPSTGASKSK
   MLRGPGPGLLLLAV
   RQAQQMVQPQSPVA
                           V
                            SQSKPGC
                                        Y D
       QQWERTY
                                        R
                                          G
                                           F
                                             Ν
                                               С
                  L
                    GNA
                         L
                           V
                            C
                                С
                                  Y
                                   GGS
                           Т
                            Y R V
                                      Y
                                 GDT
                                        E
                                          R
20
                    Y
                      T
                       G
                         N
        E E
           Т
             C
               F
                 D
                  K
                           С
                                ANRCHE
           I G A
                G
                  R G
                     R I
                         S
                            T
                              Ι
        T
          С
                                 CLGNG
                                          К
                                            G
                  Т
                    G
                      G
                       Y M
                           L
                            E
                              С
                                V
        WRRPHE
                                             Q
           CFDHAAGT
                         s y v v
                                GE
                                   T W
                                       EKP
                     RΙ
                         T
                           С
                            T
                              S
                                R
                                  N
                                   R C
                                       N
                                           D
           LGEG
                  S G
       С
        T C
                                           R G
25
                     R G
                         N
                           L
                             L
                              Q
                                С
                                  I C T
                                       G N
                                          G
           W S
               KKDN
                  s s G s
                         G
                          Ρ
                            F
                              Т
                                DVRAA
                                          Y
          S
            V
             Q
               T
                 Т
          YGHCVTDSGV
                                           Т
                           VYSVGMQWLK
       ₽
             L \ G \ N \ G \ V \ S \ C \ Q \ E \ T \ A \ V 
                                       G G N
                                             N
       С
                                T
                                  QTY
          С
          TYNDRTDST
                         Т
                                   D Q
                                       K
                           s
                             N
                              Υ
                                E
                                  Q
       PF
                                             Y
30
       V
        QTRGGN
                  S
                    N
                      G
                       Α
                         L
                           С
                             Н
                              F
                                Ρ
                                  F
                                   L
                                     Y
                                       NN
                                          Н
                                           N
                         T
                                   DOKF
                                          G
                                           F
               MKW
                       T
                           Q
                            Ν
                              Υ
                                D
                                  Α
                    С
                      G
       G
            D
             N
             TNEGV
                      MYR
                           Ι
                             G D
                                Q
                                  WDK
                                       QHDMG
       E
        I
          С
            T
                                QLRD
                                          I V
                              S
                                       Q
                                        С
          GNGRGEWT
                       С
                         I
                           Α
                             Y
       C
       NDTFHKRHEEGHM
                             L
                              N
                                С
                                  T C
                                     F
                                       G O
                                          G
                                       È
                                              H G
35
         DQCQDSE
                      G
                        Т
                         F
                           Y
                             Q
                              I
                                G
                                  D
                                   S
                                     W
                                        К
                                          Y
                                            V
       v
                    Т
                      W H C
                              L
                                  Т
                                   Y P
                                       S
                                        SSGP
                    Ε
                           Q
                             Ρ
                                Q
             RGI
                  G
                  SHPIQWNAPQPSHISKYILRW
        T
          P
            S
             Q
               P N
       Ε
       VSIPPRNL
                                                [SEQ ID NO: 1]
```

or a natural variant thereof and a polynucleotide which encodes fibronectin.

A still further aspect of the invention provides a polynucleotide which is capable of hybridising to a polynucleotide which encodes fibronectin but not a polynucleotide which encodes the polypeptide whose sequence is

```
5
   NLVATCLPVRASLPHRLN
   MLRGPGPGLLLLA
                         v
                           QC
                              LGTAVPSTGASKSK
                         A V
        QQMV
               QP
                  Q S
                      Ρ
                       ν
                            s
                              Q S
                                 ΚP
                                     GC
                                        Y
                YLGNALVCT
                                CY
                                      S
           ERT
                                   GG
                                        R G
                                            F
        Q
          W
   PEAEE
               FDKYTGNTYRVGDTYERP
           T C
           IGAGRGRISCTIANRCHE
        T C
                                          GG
   G D T W R R P H E T G G Y M L E C V C L G N G K G E W T C K
     I A E K C F D H A A G T
D C T C L G E G S G R I
                         S
                           Y
                            v
                              v
                                GETWEKP
                                             QGWMM
                         T
                           С
                            T
                              S
                                RNR
                                     С
                                      N
                                        D Q
               KKDNRGNLLQ
15
        D T
           W S
                                C
                                 I C
                                     Т
                                       G N
                                          G
                                            R G
     ΙG
           V Q T T S S G S G P F T G H C V T D S G V V Y S
                                DVRAAV
        T S
                                          Y
                                V G M
                                     QWLKT
YGGNS
     L C T C L G N G V S C Q E T A V L P F T Y N D R T D S T T S N Y
                                T Q T
                         T
L
                           S
C
                                E Q
                                   DQK
                                        Y
                                          S
                       Ā
                      G
                            H F
                                P
20
      ٧
        Q
          T
            R G
               G
                N
                  S N
                                  F
                                   L
                                       Ν
                                        N
                                          Н
                    CGT
                         T
                           QNYDAD
        RRDNMKW
                                     QKF
   SEG
                                          G
                                            F
           TTNEGVMYRIGDQWDK
   HEE
        ΙC
                                      QHDMGHMMR
                         IAYSQLRDQC
   CTCVGNGRGEWTC
                                          IVDD
   NVNDTFHKRHEEG
                         HMLNCTCFGQGRGRWKC
25
      VDQCQDSE
                      G
                       T
                         F
                           Y
                             QI
                                GDSW
                                      ΕK
                                          Y
                    T
                                            V
                                              H G
                                                 ٧
                    EWHCQ
                            P
                              L
                                   Y
      Y
        С
          Y
            G
             R
               G
                Ι
                  G
                                Q T
                                     Ρ
                                       S
                                        S
                                          S G
                                             Ρ
                                               V
               PNSHPIQWNAPQPSHISKYILRW
     T E
        Т
          P
           S Q
          I P P
               RNL
```

[SEQ ID NO: 1]

30 or a natural variant thereof.

A yet still further aspect of the invention provides a polynucleotide which is capable of hybridising to a polynucleotide which encodes the polypeptide which encodes the polypeptide whose sequence is

35 NLVATCLPVRASLPHRLN MLRGPGPGLLLLAV Q CLGTAVPSTGASKSK QPQSPVAV $s \ Q \ s \ K \ P \ G \ C \ Y \ D \ N$ QQMV C Y G G S R V G D T Y E ERTYLGNALV С T QW 40 ΚY T G N TY R E С F D С I G GRGRISCTI AN RCHE Α G G TWRRPHETGGYMLEC V C LGNGK $\hbox{\tt I} \hbox{\tt A} \hbox{\tt E} \hbox{\tt K} \hbox{\tt C} \hbox{\tt F} \hbox{\tt D} \hbox{\tt H} \hbox{\tt A} \hbox{\tt A} \hbox{\tt G} \hbox{\tt T} \hbox{\tt S} \hbox{\tt Y} \hbox{\tt V}$ GETWEKP LGEGSGRITCTSRNRCNDQ T C D C V Q T C D 45 L D W S K K D N R G N L С Т G N R R AA Y V T S S G S GPF V R H T S Q T Ρ Ρ Р Y G H c v Т DSGVVY s v $\mathsf{G}\ \mathsf{M}\ \mathsf{Q}\ \mathsf{W}\ \mathsf{L}$ K Q NGVS CQETA T С V GGN LCT LG Q Т V L P F T Y N D R T D S T T S N Y E Q D Q K Y S F C T V L V Q T R G G N S N G A L C H F P F L Y N N H N Y T 50

```
S E G R R D N M K W C G T T Q N Y D A D Q K F G F C P M A
         NEGVMYRIGDQWDK
                             QH
    ICT
        T
                                D M
    VGNGRGEWTCIAYSQLRD
                             Q
                               CI
NVNDTFHKRHEEGHMLNC
                         TCFGQGRGRWK
                   YQIGDSWEKYVH
DPVDQCQDSETGTF
              EWHCQP
     YGRG
           ΙG
                      LQT
                           Y P
                             S
                               S
                                SGP
        Q P N S H P I Q W N A P Q P S H I S K Y
  E T
     P S
RPVSIP
        PRNL
              GY
```

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[SEQ ID NO: 1]

or a natural variant thereof but not to a polynucleotide which encodes fibronectin.

Such polynucleotides can be designed by reference to Figures 1 and 2 and the known sequence of fibronectin (Kornblihtt et al (1985) EMBO J. 4, 1755-1759), and may be synthesised by well known methods such as by chemical synthesis or by using specific primers and template, a DNA amplification technique such as the polymerase chain reaction. The polynucleotide may be any polnucleotide, whether DNA or RNA or a synthetic nucleic acid such as a peptide nucleic acid, provided that it can distinguish polynucleotides which encode MSF and fibronectin as said. It is particularly preferred if the polynucleotide is an oligonucleotide which can serve as a hybridisation probe or as a primer for a nucleic acid amplification system. Thus, the polynucleotide of this aspect of the invention may be an oligonucleotide of at least 10 nucleotides in length, more preferably at least 14 nucleotides in length and still more preferably at least 18 nucleotides in length.

It is particularly preferred that the polynucleotide hybridises to a mRNA (or cDNA) which encodes MSF but does not hybridise to a mRNA (or cDNA) which encodes fibronectin.

It is also particularly preferred that the polynucleotide hybridises to a mRNA (or cDNA) which encodes fibronectin but does not hybridise to a

mRNA (or cDNA) which encodes MSF. The nucleotide sequence of MSF cDNA is disclosed herein and the nucleotide sequence of fibronectin is known (for example, see Kornblihtt et al (1985) EMBO J. 4, 1755-1759). The skilled person can readily design probes which can distinguish MSF and fibronectin mRNAs and cDNAs based on this information. Differences between MSF and fibronectin include a 45 bp deletion from the first type II fibronectin repeat module in MSF, and the unique tail present in MSF.

Preferably, the polynucleotides of the invention are detectably labelled. For example, they may be labelled in such a way that they may be directly or indirectly detected. Conveniently, the polynucleotides are labelled with a radioactive moiety or a coloured moiety or a fluorescent moiety or some other suitable detectable moiety. The polynucleotides may be linked to an enzyme, or they may be linked to biotin (or streptaridin) and detected in a similar way as described for antibodies of the invention.

A further aspect of the invention provides a method of diagnosing cancer the method comprising detecting in a sample from the person to be diagnosed the presence of a polypeptide whose sequence is

NLVATCLPVRASLPHRLN MLR G PGPG L L L L Α V Q C L G TAVPSTGAS 0 0 М V 0 Р Q S Ρ V A V S Q S K P G Y G 25 G L V С Y N A L Т С Y GG G F Q W Ε R Т S R Ν C F D K Y T G N Т Y R v G D T Y E Ε Ε Т Ε R R Ι S Т Т С Ι G Α G G С I Α N R C Н E T G Y M L W R R Ρ Н Ε G E С V С L G DHAA G T S G E T WE K С F Y V V ΕK 30 T T S CLG Ε G S G R Ι С R N R C N D С T K K D Ν R G N L L G D Т W S Q F S s G S G P Q T Т D V R Α Α v GH С Т D S G V V Y S ٧ GMQ W Ρ Ρ Y L K v s Т С N G С QE T A V T YGG N S N М C L G 0 Т 35 DRTDS Т T SNY QDQK Ρ F Т Y N Ε NSNGAL ٧ ОТ R G G CHF ₽ F LYNNH EGRRDNMKWCGTT QNYDADQKFGFC P EEICTTNEGVMYRIGDQWDKQHDMG HMMR T C V G N G R G E W T C I A Y S Q L R D Q C I V D D I

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```
NVNDTFHKRHEEGHMLNCTCFGQGRGRWKC
DPVDQCQDSETGTF
                   Y Q I G D.S
                           WE
                              KYVHG
     YGRGIG
             EWHCQPLQTYP
                             S
                              S
                                SG
                                   Ρ
                                    V
        Q P N S H P I Q W N A P Q P S H I S K Y İ L R W
    Т
     Ρ
      S
RPVSIPPRNLGY
```

[SEQ ID NO: 1]

or a natural variant or fragment thereof using a reagent which can distinguish said polypeptide from fibronectin.

A still further aspect of the invention provides a method of determining susceptibility to cancer the method comprising detecting in a sample derived from the person to be tested the presence of a polypeptide whose sequence is

```
15
   NLVATCLPVRASLPHRLN
   MLRGP
         GPGLLLLA
                    V
                      Q
                       CLGTAVPS
               QSPVAVSQSKPGCYDN
                                     GKHY
    VMQQAQ
            QΡ
      QWERTYLGNALVCTCYGGSRGF
   PEAEETCFDKYTGNTYRVGDTYERPKDSMI
20
   W D C T C I G A G R G R I S C T I A N R C H E G G Q S Y K I
                   YMLEC
                          VCLGNG
    DTWRRPHETGG
                                  K
                   T
                    SYVV
                          GETWE
    IAEKCF
            DHAAG
                                ΚP
         LGEGSGRITCTSRNRCND
                                  QDT
       T C
      DTWSKKDNRGNLLQCICT
                               G N
    ΙG
25
   ERHTSVQTTSSGSGPFT
                          DVRAAVY
   QPPPYGHCVTDSGV
                      VYSVGMQWLKT
                                     Q
         LGNGV
                S
                 С
                   Q
                    E
                      T
                       Α
                          Т
                               G
   MLC
      T C
                           Q
                             T
                                 G
                                  N
         YNDR
               T
                D
                 S
                   Т
                    T
                      S
                       N
                         Y
                          E Q
                            DQKY
                                  S
                      CHFPFLYNNHNY
               S N G A L
           GGN
     V
        Т
         R
30
       RRDNMKWCGT
                    T
                      QNYDADQKFGF
     G
           TNEGVMYRIGDQWDKQHDMGHMMR
   HEEICT
                    IAYSQLRDQCIVDD
     CVGNGRGEWTC
   NVNDTFHKRHEE
                   G
                    H M L
                         N
                           Τ
                              F
                               G
                                 Q
                                  G
                                   R G
                    F
                      Y
                         I G
                           D
                             S W
                               E
           Q D S
               E
                Т
                  G
                   Т
                       Q
                                 K
                                  Y
                                    v
               GEWHCQP
35
     Y
           RGI
                        LQTYPSSSG
       С
        ΥG
           Q P N S H P I Q W N A P Q P S H I S K Y I L R W
       T P
         S
     Ε
     VSIPPRNLGY
```

[SEQ ID NO: 1]

or a natural variant or fragment thereof using a reagent which can distinguish said polypeptide from fibronectin.

A still further aspect of the invention provides a method of determining the likely outcome of a patient with cancer the method comprising

detecting in a sample from the patient the presence of a polypeptide whose sequence is

```
NLVATCLPVRASLPHRLN
                           A V
                               QCLGTAVPSTGASKSK
                   LLLL
             G
               P
                 G
                       S P
                           VAVSQSKPGCYDNG
                   P
                     Q
               v
             М
                   YLGNALVCTC
                                       YGGSRG
           WERT
         Q
                           GNTYRV
                                       GDTYERPK
               CFDKYT
    PEAEET
           CIGAGRGRISCTIA
                                       N R C
                                             H E
                                                 G
                                                  G
       СТ
    GDTWRRPHETGG
                           Y
                             ML
                                 E
                                   С
                                     v
                                       С
                                           G
                                               G
10
                               Y
                                 v
                                   v
                         G
                           Т
                             S
                                     G
                                       E
                                         Т
                                           W
                                             E
                                               K
                                                 P
         EKCF
                 DHAA
       Α
                         R I
                     S
                       G
                             T
                               С
                                 Т
                                   S
                                     R
                                       Ν
                                         R
                                           Ç
                                               D
         Т
            С
             L
               G
                 Ε
                   G
                   KDNR
                           G
                             N
                               L
                                   Q
                                       Ι
         D
            Т
             W
               S
                 K
      I
        G
                                   T
                                     D V
                                           Α
                               ΡF
                                         R
             v
               QT
                   T S S G
                           S
                             G
                                             Α
                                                 Y
        Н
         Т
            S
                   VTDSG
                               V
                                 Y
                                   s
                                           QW
                                               L
                                                 К
             GHC
                             V
                                     V
                                       G
                                         М
                                                     Q
15
            Y
                           Q
T
        С
           С
             L G N
                   G
                     V
                       s
                         С
                             E
                               Т
                                 Α
                                   v
                                       Q
                                             G
         T
                                   Y
                                         D
                                           QK
                                               Y
                                                   F
                               S
                                     E
                                                 S
                         S
                             Т
                                 N
                                       Q
        Р
            Т
             Y
               N
                 D
                   R
                     T
                       D
                     SNGAL
                               C H F
                                     ₽
                                       F
                                         L
                                           YNNH
            Т
                 G
                   N
          Q
             R
               G
                  \begin{smallmatrix} M & K & W & C & G & T & T & Q & N & Y & D & A & D & Q & K \\ N & E & G & V & M & Y & R & I & G & D & Q & W & D & K & Q \\ \end{matrix} 
                                           QKF
        G
           R D
         R
               N
                                               H D
20
         I
           С
             T
               Т
            GNGRGEWTC
                             IAYSQLRD
                                             Q C
               HKRHEE
                           GHMLN
                                     С
                                         С
                                           F
                                               0
      VNDTF
                                     G
                                       D S
                                           W
                                             Ε
                                               K Y
                                                   V H
               QDSE
                       T
                         G
                           Т
                             F
                               Y
                                 Q
                                   I
        V D
           0 C
                       EWHCQP
                                       T Y
                                           P S
                                                   G P
                                   L Q
                                               s s
              G
               R
                 G
                   ΙG
               Q P N S H P I Q W N A P
                                       QP
25
        E T
            P
             S
        VSIPPRNLGY
```

[SEQ ID NO: 1]

or a natural variant or fragment thereof using a reagent which can distinguish said polypeptide from fibronectin.

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Preferably, the reagent which can distinguish MSF from fibronectin is an antibody as disclosed herein. The use of antibodies to detect specific polypeptides in samples is well known. For example, they can be used in enzyme-linked immunosorbend assays (ELISA) or they may be used in histopathological analysis. It is believed that the presence of MSF indicates an elevated risk of cancer.

MSF may be conveniently measured in suitable body fluids such as serum or urine, or in extracts of tissue, or in the medium used to culture patient derived cells *in vitro*.

The measurement of MSF is believed to be useful in a number of cancers as discussed above. Antibodies may be used to detect MSF in tissue sections by immunolocalisation. Sub-populations of MSF-producing fibroblasts are present in the normal adult (Irwin et al (1994) J. Cell Science 107, 1333-1346; Schor et al (1994) pp 277-298 in Mammary Tumorigenesis and Malignant Progression, Dickson, R. and Lippman, M. (eds), 1994, Kluwer Academic Publishers.

It will be appreciated that, as well as the MSF polypeptide being measured using the methods described herein in diagnosis or prognosis or determination of susceptibility to cancer, it may be desirable to detect MSF mRNA in a suitable sample or it may be desirable to detect any changes in the fibronectin gene which are associated with the production of MSF. Mutations in the MSF cDNA or fibronectin gene may be detected using methods well known in the art.

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Thus, a further aspect of the invention provides a method of determining susceptibility to cancer the method comprising detecting in a sample derived from the person to be tested the presence of a polynucleotide encoding a polypeptide whose sequence is

NLVATCLPVRASLPHRLN MLRGPGPGLLLLAV QCLGTAVPSTGASKSK Q P Q S ₽ V Α ٧ S QSKPG QQMV 25 ER Т Y L GNALV СТ C Y GGS R G NTYRVGDT С F D K Y Т G Y ERP Ε T G R GRIS CTIANRCHE GG С С Т Ι G Α G GYMLECVCLGNGK Т Ρ H E T W R R D H A A G T S Y V V G E T W E K P I A E K С F G E G S G R I T C T S R N R C N D Q D T 30 С C DTWSKKDNRG N L L С I C Т GNG R Q G S G S G Ρ F Т D V R Α RH T S V QTT S Α G S S Y G H С V T D V V Y V G М W L PΡ Ρ G V S С Q E T Α V Т Q T Y GG S С T С L G N S Т T 35 Y D R Т D S N Y EQDQKY F Ρ F Т N SNGALCHF Р F LY V Т G G N Q R F GTTQNYDADQKF EGRRDNM KWC G MYRĪGDQWDKQHDMGHM $\hbox{\tt H} \ \hbox{\tt E} \ \hbox{\tt E} \ \hbox{\tt I} \ \hbox{\tt C} \ \hbox{\tt T} \ \hbox{\tt T} \ \hbox{\tt N} \ \hbox{\tt E} \ \hbox{\tt G} \ \hbox{\tt V}$ CTCVGNGRGEWTCIAYSQLRDQCIVDDITY

```
N V N D T F H K R H E E G H M L N C T C F G Q G R G R W K C
D P V D Q C Q D S E T G T F Y Q I G D S W E K Y V H G V R Y
Q C Y C Y G R G I G E W H C Q P L Q T Y P S S S G P V E V F
I T E T P S Q P N S H P I Q W N A P Q P S H I S K Y I L R W
R P V S I P P R N L G Y
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[SEQ ID NO: 1]

or a natural variant or fragment thereof using a reagent which can distinguish said polynucleotide from a polynucleotide encoding fibronectin.

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A still further aspect of the invention provides a method of determining the likely outcome of a patient with cancer the method comprising detecting in a sample from the patient the presence of a polynucleotide encoding a polypeptide whose sequence is

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NLVATCLPVRASLPHRLN
    MLRGPGPGLLLLAV
                                 QCLGTAVPSTGASKSK
                                V S
V C
                                     Q S
                                         K P
                  QPQSPVAV
            QMV
            WERT
                    YLGNAL
                                       С
                                         Y
                                           G
                                             G
                                                 R
                                     R V
                            \mathsf{G}\ \mathsf{N}\ \mathsf{T}\ \mathsf{Y}
                                         G D T
                                               Y
                                                 E R P
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                    DKY
                          T
            Ε
              Т
                  F
                    G R G R I S C T
                                     IANRCHE
              Ι
                G A
                            YMLE
                                     С
                                       V
                                             GNGK
          W
            RRPHETGG
                                         C L
                FDHAAGTSYV
                                     VGETWEKP
                                                     Y
            КС
      IAE
                G E G S G R I T C T S R N R C N D
                                                   Q
              L
          T
            С
            TWSKKDNRGNL
                                   L
                                     Q
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S
V
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                                       С
                                         I C
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          D
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                                             Α
            S
Y
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C
                    T
V
                        S G
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                Q
                      T D S S N G
                                             Q
Y
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                                   Y
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                                 T A
S N
C H
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I G
                            Q E
T T
A L
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Y R
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                                         QT
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                                                 G N
          T
              L
                                     Y
F
Y
                                         Q D
F L
                                             Q K
Y N
                                       E
                    R
                N
                  D
                                       P
            T
                    N
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              RGG
                         C
V
                                         A D
                    K W
E G
                                       D
                                             Q
                                                 F
                                                     F
          RRDN
                  М
                                               K
                           М
                                     D
                                             K Q H D
                                       QWD
                Т
                  N
              N G R G E W T
F H K R H E E
                             Q
G
                                       Q
                                         L R
                                             D
            G
                                           С
                                         T
                                             F
                                                 QG
                                                     R
      VNDT
                                                 ĸ
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                      ETG
                             T F Y Q
                                     I G
                                         D
                                           S
                                             W
                                               Ε
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                Q D
                    S
                                           . Y
                                               S
                                                   S
                                                      G
            Y
              G
                R G
                    I
                       G
                        Ε
                           WHC
                                 QP
                                     L
                                       Q
                                         T
                                             Ρ
                                                       Ρ
                             IQWNAP
                                         QP
                                             S
                                               Н
                                                 I
                                                   S
            PSQPNS
IPPRNL
                        H P
        E T
            PS
                         G
```

[SEQ ID NO: 1]

or a natural variant or fragment thereof using a reagent which can distinguish said polynucleotide from a polynucleotide encoding fibronectin.

Preferably, the reagent which can distinguish the polynucleotide encoding MSF from the polynucleotide encoding fibronectin is a suitable polynucleotide as disclosed herein. Methods of detecting specific nucleic acids in a sample are well known in the art. For example, in situ hybridisation methods which detect mRNA may be used, and northern blotting methods may be used. Dot blots, slot blots and Southern blots may also be used.

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Thus, it can be seen that the reagents used in the above methods may be used in the manufacture of a reagent for diagnosing cancer.

It will be appreciated that the antibodies of the invention, and the polynucleotides of the invention, which can distinguish MSF and fibronectin (particularly those which recognise MSF or a nucleic acid encoding MSF, but not fibronectin, or a nucleic acid encoding fibronectin) are useful packaged into diagnostic kits containing said antibodies or polynucleotides and other reagents such as means for labelling the said antibodies or polynucleotides.

The invention also includes a number of therapeutic applications, for example chemoprevention and chemotherapy.

Chemoprevention includes the neutralisation of MSF activity and/or the suppression of inappropriate MSF expression in individuals deemed to be at risk of cancer due to inappropriate MSF production. It may also be desirable to administer inhibitors of MSF. Antibodies directed at MSF may act as inhibitors.

Chemotherapy includes the use of anti-MSF antibodies to target coupled cytotoxins to sites of inappropriate MSF production, and the use of MSF inhibitors as mentioned above.

Antibody-targeted cytotoxins are well known in the art and include antibodies linked to a directly cytotoxic moiety such as ricin or a toxic drug; and antibodies linked to an indirectly cytotoxic moiety such as an enzyme which is able to convert a non-toxic prodrug into a toxic drug. In the latter case, the prodrug as well as the antibody-linked enzyme is administered to the patient.

It is useful to measure MSF in wound fluids since this information may be relevant in terms of predicting the efficiency of the subsequent healing process, including the propensity of the scar. The amount of MSF in wound fluids may be measured using, for example, an MSF-selective antibody of the invention.

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Inappropriate expression of MSF may be a feature of several pathological conditions characterised by inflammation, such as rheumatoid arthritis. The measurement of MSF in associated body fluid, such as synovial fluid, may be of clinical utility; other pathological conditions of relevance in this context include fibrotic and periodontal disease.

MSF is believed to be involved in the migration of cells, especially fibroblasts any, in particular, the migration of cells may take place within the wound.

Thus, a further aspect of the invention provides a method of modulating cell migration the method comprising administering an effective amount of

a polypeptide of the invention to the site where modulation of cell migration is required.

Typically, the cell whose migration is modulated is a fibroblast. Typically, MSF stimulates the migration of cells such as fibroblasts. Preferably, the site where modulation of cell migration is required is a site within a mammalian body, such as the body of a horse, pig, cow, sheep, cat, dog and the like. Most preferably it is a site within a human body. It is preferred if the site within the body is the site of a wound.

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A further aspect of the invention provides a method of healing a wound the method comprising administering to the locality of the wound an effective amount of a polypeptide of the invention.

The invention also includes a method of preventing scarring by administering to the locality of the site where scarring is believed to be likely an effective amount of an MSF polypeptide as described herein or a suitable fragment or variant. Preventing or reducing scarring may be part of the wound-healing process. The MSF polypeptide as described herein or a suitable fragment or variant is believed to be useful in preventing or reducing scarring because it reduces hyaluronic acid formation.

It is preferred if the polypeptide administered is a recombinant polypeptide expressed in a eukaryotic host.

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The MSF polypeptide may be administered to the site of cell migration or wound healing by any suitable means. Conveniently, the polypeptide is administered topically. It is particularly preferred if the polypeptide is incorporated within an applied wound dressing such as a collagen mesh.

Dressings which are suitable for the incorporation of the polypeptide of the invention are well known in the art and many are commercially available.

Other formulations might involve the incorporation of MSF into an ointment, paste, gel, cream (or equivalent) designed for topical application.

The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. Such methods include the step of bringing into association the active ingredient (polypeptide of the invention) with the carrier which constitutes one or more accessory ingredients. In general the formulations are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

Formulations in accordance with the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets, each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution or a suspension in an aqueous liquid or a non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be presented as a bolus, electuary or paste.

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Formulations suitable for topical administration in the mouth include lozenges comprising the active ingredient in a flavoured basis, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient

in an inert basis such as gelatin and glycerin, or sucrose and acacia; and mouth-washes comprising the active ingredient in a suitable liquid carrier.

It should be understood that in addition to the ingredients particularly mentioned above the formulations of this invention may include other agents conventional in the art having regard to the type of formulation in question, for example those suitable for oral administration may include flavouring agents.

10 Application of gene therapy techniques may provide a means of controlling MSF expression.

Any suitable amount of the polypeptide of the invention may be administered. By "suitable amount" we mean an amount which gives the desired biological response and that does not lead to any significantly undesirable effects such as toxicity or the like. Small quantities of MSF, for example less than 1 µg, may be effective. It is preferred if superficial wounds, such as those to the skin, are treated by the method of the invention.

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The invention will now be described in further detail with reference to the following Figures and Examples wherein:

Figure 1 shows the entire nucleotide sequence of the 2.1kb insert in clone $pMSF1\alpha$ which contains the MSF cDNA. The start and stop codons are underlined.

Figure 2 shows the translation of the cDNA sequence shown in Figure 1 and the alignment of the peptide sequence with that of the gelatin-binding

domain of fibronectin. The start and end of the MSF polypeptide are indicated by vertical bars and arrows.

Figure 3 shows the peptide sequence of MSF (as encoded in the pMSF1 α clone) according to its domains. The sequence of pMSF1 α is shown grouped according to its domains (cf analysis of fibronectin from Kornblihtt et al (1985) EMBO J. 4, 1755-1759). Residues are numbered and have been aligned to give optimal homology by introducing gaps (indicated by ^). Identical residues within a type of homology are indicated by a box (A), and stop codons are designated by asterisks (*). Deleted amino acids are indicated by dashed lines (-), and the IGDS sequence is underlined.

Figure 4 shows a diagrammatic comparison of fibronectin and MSF.

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Figure 5 shows a diagrammatic model of MSF showing the positions of the IGD-containing sequences (ie. IGDT, IGDS and IGDQ) within the domains.

20 Example 1: Cloning and sequence analysis of pMSF1 \alpha, a clone encoding MSF

A cDNA library was constructed using mRNA extracted from a human foetal fibroblast cell line, MRC5-SV2, in the vector λ ZapII.

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A primer based on peptide sequence from the gelatin-binding domain (GBD) of fibronectin was used together with a vector primer in the polymerase chain reaction (PCR) to amplify a fragment of 1.2kb. Sequence analysis showed a strong homology to GBD for most of the

fragment. Clear differences included an internal deletion of 45bp, and a 3' unique sequence of 175bp.

The 3' unique sequence was used as a probe for screening the library, using the digoxigenin-labelled system. Positive plaques were picked for further analysis by secondary and tertiary screening, followed by *in vivo* excision of the pBluescript™ phagemid containing the cloned insert.

A plasmid containing an insert of 2.1kb, named pMSF1α, was sequenced by the Sanger-dideoxy method, using a progressive priming approach, and the sequence was assembled into a single contain using the Fragment Assembly System of the Daresbury/Sequet series of programs.

The entire nucleotide sequence of the 2.1kb fragment is shown in Figure 1.

Translation of this sequence and alignment of its peptide sequence with that of the gelatin-binding domain of fibronectin was achieved using the Fasta program (Daresbury/Sequet), and is shown in Figure 2.

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Figure 3 shows the peptide sequence of pMSF-1 α grouped according to its domains.

Other cDNA clones encoding MSF may be readily obtained and sequenced using methods well known in the art and probe derived from the Figure 1 sequence, in particular probes which distinguish MSF from fibronectin.

Example 2: Demonstration of the presence of MSF-secreting fibroblasts in sections of breast cancer, but not normal breast sissue

In situ hybridisation using a riboprobe based on the unique coding region for the unique C-terminus of MSF demonstrates the presence of MSF-secreting fibroblasts in sections of breast cancer, but not normal breast tissue.

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Suitable riboprobes contain the entire unique nucleotide sequence of MSF- 1α (position 1953-2147), and may include up to 10 bases upstream and contained within the fibronectin sequence (position 1943-2152). This ensures high specificity towards MSF- 1α , whilst allowing the use of a probe of longer length. A digoxigenin-labelled riboprobe containing a major portion of the unique sequence (position 1974-2147) is used. This region was selected on the basis of the position of convenient restriction sites.

Example 3: Monoclonal antibodies which are specific to MSF and do not cross-react with fibronectin

Monoclonal antibodies are raised using any of the currently available standard procedures. The immunogen is a synthetic peptide based on the 10 amino acid unique tail of MSF or is based on the peptide sequences:

ISKYILRWRPVSIPPRNLGY; [SEQ ID NO: 3]; or QQWERTYLGNALVCTCYGGSR; [SEQ ID NO: 4]; or PCVLPFTYNDRTDSTTSNYEQDQ; [SEQ ID NO: 5]; or TDHTVLVQTRGGNSNGALCH; or [SEO ID NO: 6]; or VGNGRGEWTCIAYSQLRDQCI [SEQ ID NO: 7]

Example 4: Genomic PCR and FISH studies

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Objective: To obtain information regarding the sequence of the genomic 5 MSF gene regarding (i) its relationship to fibronectin, and (ii) chromosomal location.

Background: The 5' upstream untranslated sequence of the cloned MSF cDNA is identical to that of fibronectin, thereby strongly suggesting its close relationship to the fibronectin gene (note: such upstream untranslated regions are virtually never identical between two genes as there is no selective pressure. This inference is in apparent conflict with the "uniqueness" of the 3' end of the MSF cDNA which codes for a 10 amino acid polypeptide and also contains a contiguous untranslated region containing several stop codons).

Methods and Results: Two PCR reactions were established: one at the extreme 5' untranslated region of fibronectin (FN)/MSF and the other at the extreme 3' region of MSF which encompassed the 175bp unique sequence. Reactions were carried out using DNA purified using the Qiagen Blood kit. Sequence analysis of the resulting amplicon revealed that the 175bp "unique" sequence was contiguous with the fibronectin sequence.

Experiments were then carried out in order to obtain initial data regarding the genomic location of the 3' unique sequence. This was accomplished by selecting clones from the human PAC library (obtained from HGMP) using the above 2 PCR approach. Secondary and tertiary screening lead

to the identification of on which produced products from both PCR reactions. This clone was approximately 70-110 kb in size.

The isolated clone was next subjected to restriction digestion (BamHI and KpnI) and the fragments subcloned into pBluescript and analysed using our 2 PCR approach. Two positive clones were identified: clone B3(2) is 20 kb and can generate both the 5' and 3' fragments, thereby indicating that it contains the entire MSF genomic sequence. The other clone, K5(5) is 7 kb and only contains the 3' unique sequence.

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We have used both clones for FISH analysis of the human genome. Our data unambiguously indicate that MSF maps to chromosome 2 region q35. Note: this is within the fibronectin gene, which is located on chromosome 2q34-36.

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Conclusions: The FISH analysis clearly indicates that the gene coding for the MSF "unique" sequence is contained within the fibronectin gene. These results indicate that MSF is a novel "mini" splice variant of fibronectin. The genomic fibronectin gene is very large indeed and has still not been fully sequenced. To our knowledge, this is the first report of the unique sequence. The absence of the unique sequence in all previously identified isoforms of fibronectin (which are all in excess of 220 kDa compared to 70 kDa for MSF) indicates that it is spliced out of these molecules.

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This information is of relevance for several reasons. Firstly, all previously described splice variants of fibronectin have molecular masses in the region of 225 kDa compared with only 70 kDa of MSF. This small size is totally unexpected and prompts us to refer to MSF as a novel

"mini" splice variant of fibronectin. Secondly, all known splice variants of fibronectin involve the inclusion/deletion of entire type III repeats or variable regions of the IIICS region (all of which occur at a considerable distance downstream of the termination of MSF, which does not contain any known splice site). Finally, as the unique 3'-sequence of MSF was not hitherto identified, it was not possible to predict that MSF was indeed a splice variant of fibronectin until the above data was obtained from genomic DNA.

10 Example 5: Recombinant MSF expression

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Objective: To express recombinant human MSF (rhMSF) in 3T3 cells.

3T3 cells were transfected using the Methods and Results: Lipfectamine/Plus system (Gibco), according to the manufacturer's The plasmid used was pcDNA3.1/hisB/lacZ. instructions. sequence contained a sequence encoding a his tail fused to the human MSF cDNA sequence so that a fusion protein with a his tail is expressed. This Transfectants were facilitates purification of the expressed protein. isolated by their selective growth in medium containing 418. One liter of conditioned medium produced by the transfected cells was collected and the fraction containing all the migration stimulating activity obtained by doing a 0-20% ammonium sulphate precipitation. The pellet was resuspended in buffer and the his-tagged rhMSF purified by passage through a ProBond column (Invitrogen) column, all done in accordance with manufacturer's instructions. Approximately 250 µg of rhMSF were collected from the starting material. The purified protein resulted in a single band of approximately 70 kDa in SDS PAGE. This protein stimulated the migration of target adult fibroblasts and was active at

concentrations between 1 pg/ml to 10 ng/ml (ie in precise agreement with previously published data regarding the dose-response of MSF purified from fetal fibroblast conditioned medium).

5 Example 6: Anti-MSF antibody production

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Objective: To generate polyclonal antibodies to MSF.

Methods: Rabbits were immunised with a 15-mer synthetic peptide based on the C-terminus of MSF: note, this contains the entire 10 amino acid unique sequence and the contiguous 5 amino sequence of fibronectin. The synthetic peptide was coupled to keyhole limpet haemocyanin (KLH) carrier and used to immunise two rabbits with the following protocol: first injection of 10 mg and second injection of 5 mg three weeks later. Serum was collected six weeks after the first injection and purified IgG shown to recognise the synthetic peptide in both dot and Western blots.

Results: We have used the antibody for both Western blots and immunohistochemistry. The former application has (i) confirmed that rhMSF is recognised by the antibody, and (ii) demonstrated that fetal, but not adult, fibroblasts produce a 70 kDa molecule which is recognised by the antibody and expresses migration stimulating activity when eluted from the PAGE gels.

Polyclonal antibodies were generated against a synthetic peptide incorporating the 10 amino acid "unique" MSF C-terminal sequence. This antibody recognises the unique synthetic peptide (down to 5 ng) and MSF (down to 10 ng) in dot blots; it does not recognise fibronectin or BSA at concentrations up to 4 μg. This antibody has been used to

investigate the tissue distribution of MSF; these experiments show that MSF is present in the stromal compartment of fetal skin and is not detectable in adult skin.



CLAIMS

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1. A recombinant polynucleotide encoding a polypeptide comprising the amino acid sequence

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5
   NLVATCLPVRASLPHRLN
            GPGLLLLA
                          V
                              CLGTAVPSTGASKSK
   MLRGP
                            Q
                   Q S P V A V S Q S K P G C Y D N G K H Y
       QQWERTYLGNALVCTCYGGSRGFNC
                              Y
                               R V
     EAEETCFDKYTGNT
                                   G
                                    DTYE
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                 GRGR
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            IGA
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                H E
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                               V
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                                     TWEKP
         E
          K
            С
              F
                D H
                   Α
                     Α
                 GSGRI
                          T
                            CTSRNR
                                      C N D
          С
              G
                Ε
                        G N L L
                 KDNR
                               Q
                                 С
                                   ICTGNGRGE
              S K
15
          s v
              QT
                 T S S G
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                          G
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                              F
                               T
                                 D V
                                     RAAV
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                  G
                   V
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                                        GG
                                           N
              G N
                     D S
                        T
                          Т
                            S N Y E
            Y
                DRT
                                   QDQKY
              N
                 NSNGAL
                            CHFP
                                   FLYNNHNY
       V
          T R
              G
                G
         Q
20
                     CGTT
                            QNYDADQKF
                                           G
       GRRD
              NMKW
       EICTTNEGVMYRIGDQWDKQH
                                            D M G
          \mathsf{G}\ \mathsf{N}\ \mathsf{G}\ \mathsf{R}\ \mathsf{G}\ \mathsf{E}\ \mathsf{W}
                      T
                         С
                          ΙA
                              Y
                                S
                                 Q
                                   L
       С
         V
                                     R D
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                        G
                              L
                                 C
         D
          T
            F
              HKRH
                     ΕE
                          н м
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                                     С
                                      F
                                        G
                                            G
              Q D S E T
       V
          Q C
                      G
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                          F
                            Y Q
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                                   D S
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                                        E
         D
25
          Y G
              R
                 IGEWHCQP
                               LQT
                                     YPSSSGP
       Y C
                G
                         IOWNAPOPSHI
                 NSHP
                                           SKYILRW
           Ρ
            S
              Q
                Ρ
          IPPRNLGY
```

[SEQ ID NO: 1]

or variants or fragments or derivatives or fusions thereof or fusions of said variants or fragments or derivatives.

- 2. A polynucleotide according to Claim 1, encoding a polypeptide comprising the amino acid sequence shown in Figure 2 labelled pMSF1a between positions 19 and 660, or variants or fragments or fusions or derivatives thereof or fusions of said variants or fragments or derivatives.
- 3. A polynucleotide according to Claim 1 or 2, which contains no introns.
- 40 4. A polynucleotide according to any one of the preceding claims, comprising the polynucleotide whose sequence is shown in Figure 1.

5. A polynucleotide according to any one of the preceding claims, comprising the polynucleotide whose sequence is shown in Figure 1 between positions 57 and 1982.

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- 6. A polynucleotide according to any one of the preceding claims, encoding a polypeptide which has migration stimulating factor activity.
- 7. A replicable vector comprising a polynucleotide as defined in any one of Claims 1 to 6.
 - 8. A host cell comprising a recombinant polynucleotide or a replicable vector as defined in any one of Claims 1 to 7.
- A method of making a polypeptide having the amino acid sequence 9. 15 LPVRASLPHRLN NLVATC GLLLLAV OCLGTAVPSTGASKSK ₽ G Ρ $Q \ P \ Q \ S \ P \ V \ A \ V \ S \ Q \ S \ K \ P \ G \ C \ Y \ D \ N \ G$ КН QM V YLGNALVCTCYGGSRG N
- INQQWERT EAEETCFDKYTGNTYRVGDT 20 DCTCIGAGRGR I S С T Ι A N R С Η Ε G G Y М L Ε С V С G N G К ΗE T G G L DTWRRP v T S Y GE TWEK A A G Ε К С F DН GSGRI \mathbf{T} CTSRNRCNDQDT
- LGE С Т С DTWSKKDNRGNLL T G N 25 G Q С ΙC P v s s S G F T D R Α Α $\mathtt{T} \ \mathtt{S} \ \mathtt{V} \ \mathtt{Q} \ \mathtt{T} \ \mathtt{T}$ G V v Y s V L K GHC V T D S G. G М Q W Р PΥ T Y С E T A V Т GGNS G v S Q Q T С G N
- DRTDS Т Т SNYE QDQKY SF Т Y N P F T CHFPFLYNNH N RGGNSN GAL 30 V Q T RRDNMKWCGTT QNY DADQK F G F E G ICTTNEG V M Y R Ι G DQW D K Q Н D M E E С Υ S R D Q C Ι V Т I A Q L N G R G E W T С V G
- K R H E E G H M LN C T С F G DTFH G D S W E К Y V Н G T F Y QI 35 QCQDS Ε Т G P V D GEWHCQPL Y P S S S G Ρ V Ε GI QT G R P S Q P N S H P I Q W N A P Q P S H I S K I RPVSIPPRNLG [SEQ ID NO: 1]

or variants or fragments or fusions or derivatives thereof, or fusions of said variants or fragments or derivatives, the method comprising culturing

said variants or fragments or derivatives, the method comprising culturing a host cell as defined in Claim 8 which expresses said variant or fragment

or derivative or fusion and isolating said polypeptide or variant or fragment or derivative or fusion from said host cell culture.

10. A polypeptide comprising the amino acid sequence

```
5
                CLPVRASLPHRLN
                     GLLLLAV
                                     QCLGTAVPS
     MLRGP
                G P
                                VAVS
                                          QSKPGC
                                                       Υ
                                                          D
                                                            N
                                                              G
              QMV
                       Р
                          0
                            S
                              Ρ
           Q
                     Q
                            GNALVC
                                            CYGGS
                                                       R G
                                                            F
                                         T
                E R
                     T
                       Y
                          L
                     FDKYT
                                GNTYRVGDTYERPKD
              E
                Т
                  С
10
                IGAGRGRISCTIANRCHE
              С
             \hbox{\tt W} \hbox{\tt R} \hbox{\tt R} \hbox{\tt P} \hbox{\tt H} \hbox{\tt E} \hbox{\tt T} \hbox{\tt G} \hbox{\tt G} \hbox{\tt Y} \hbox{\tt M} \hbox{\tt L} \hbox{\tt E} \hbox{\tt C} \hbox{\tt V} \hbox{\tt C} \hbox{\tt L} \hbox{\tt G} \hbox{\tt N} \hbox{\tt G} \hbox{\tt K} 
       IAEKCFDHAAGT
                                   SYV
                                          V
                                            GETW
                                                     E
                                                       K
                                                   С
              CLGEGS
                            GRI
                                   T
                                     С
                                       T
                                          SRNR
                                                     N
                                                       D
           Т
                                     L
                                                   Т
                                                          G
                                G N
                                       L
                                          Q
                                              I C
                                                     G
                W S
                     K
                       K D
                            N
                              R
                                            С
                                                       N
                                                            R
                v
                                              V R
                                                   A A
15
            T
              s
                  Q T
                       T
                         S
                            S G
                                S
                                   G
                                     ΡF
                                          Т
                                            D
              Y
                G H
                     С
                       V
                         T D S
                                G
                                   v
                                     V
                                       Y
                                          S
                                            v
                                              \mathsf{G} \; \mathsf{M} \; \mathsf{Q} \; \mathsf{W} \; \mathsf{L} \; \mathsf{K}
            P
                                                  Y
                                Q
T
                     NGVSC
                                   E T A V
                                            T
                                              Q
                                                Т
                                                     G
                                                       G
                                                          N
                                                            S
                  G
                                   T
L
                                                   Q K
Y N
                YNDRTDS
                                     SN
                                          Y
                                            E
                                              Q D
              Т
                                     С
                                       Н
                                          F
                                               F
                                                       N
                                                          Н
                                                            N
              Т
                RGGNS
                            N
                              G
                                Α
                                            P
                                                 L
            0
                                   Т
                                       N
                                          Y
                                            D A D
                                                   QKF
20
                       K W
                            С
                              G
                                Т
                                     Q
                                                          G
                                                            F
                 D
                  NM
                     N E G V
                              M Y
                                   RIGD
                                            QWDKQHDMGHMMR
                T
                  T
            Ι
              С
         С
            V G
                NGRGEWT
                                С
                                   IAYS
                                            QLRDQ
                FHKRHEEGHMLN
                                            CTCFG
                                                       QGRG
       VNDT
              QCQDSETGTF
                                     Y
                                       QIG
                                              D S
                                                   W
                                                     Ε
                                                       К
       PVD
                              W H C
                                                        S
                                                            G
25
              Y
                 G R
                     G
                       I
                          G
                            Ε
                                     QP
                                          L
                                            QT
                                                 Y
                                                   Р
                                                     S
                                                          S
                                                              Ρ
                                                                 V
         Y
            С
                                                        I
                          S H
                              Ρ
                                Ι
                                   QWNAP
                                              Q
                                                 Ρ
                                                   SH
                                                          S
                                                            K
                                                                 Ι
            Т
              Ρ
                 S Q
                     ₽
                       N
     RPVSIPPRNLG
```

[SEQ ID NO: 1]

or variants or fragments or fusions or derivatives thereof or fusions of said variants or fragments or derivatives.

- 11. A polypeptide according to Claim 10, comprising the amino acid sequence shown in Figure 2 labelled pMSF1 α between positions 19 and 660, or variants or fragments or fusions thereof or fusions of said variants or fragments.
- 12. A polypeptide obtainable by the method of Claim 9.

35

13. A polypeptide according to any one of Claims 10 to 12, which has migration stimulating factor activity.

An antibody reactive towards the polypeptide whose amino acid sequence is

```
NLVATCLPVRASLPHRLN
   MLRGPGPGLLLLAVQCLGTAVPSTGASKSK
                           V S
V C
                               Q S
T C
    R \ Q \ A \ Q \ Q \ M \ V \ Q \ P \ Q \ S \ P \ V \ A 
                                  ΚP
                                      G
5
                                  Y
                                    G
                                       S
                                         R
                                           G
          W
            ERT
                 YLGNAL
                                     G
                             Y
                               R V
                           T
                                  GDT
                                       Y
                        G N
                                         Ε
                                           R P
            T
              С
               F
                 DKY
                      T
            IGAGRGRISCT
                               I A N R C H E
          С
                                           GG
        T
                             Ε
          RRPHETGG
                        Y M L
                               cv
                                  С
                                    L
                                      GNGK
             FDHAAGTSYVVGETWEKPYQ
10
            С
     IAE
          K
            LGEGSGRITCTSRNRCNDQ
                                             D T
       С
        Т
          С
                                      T
A
        DTWSKKDNRGN
                           L
                             L
                               Q
                                 С
                                  I
                                    С
                                       G
                                         N
                                             R
   RIG
                           PF
                               T
                                 D
                                  ν
                                    R
                                         v
                        S
                         G
                                       Α
                                           Y
                                              Ρ
       Н
        Т
          S
            V
              QT
                 T
                   S
                    SG
               С
                 V
                   T D
                      S
                        G
                         v
                           V Y S
                                V
                                  G M
                                      QWL
                                           K
                                             Т
          Y
            G H
                                              Q
       Р
        Р
            L G N G V S C Q E
Y N D R T D S T T
                           TAV
                                Т
                                  Q T
                                      Y
                                       G
15
       С
        T
          C L
          T Y N D R T D S T T S N Y E Q D Q K Y S T R G G N S N G A L C H F P F L Y N N H
                                            F.
                                              C
        F
                                             N
       GRRDNMKWCG
                        D
                                  Α
                                    D
                                      QK
                                         F
                                             F
                                 QWD
                                      K
                                           D
         I C
            Т
              Т
                NE
                   G
                    V
                      М
                                       Q
                                         Н
                                             M
                                              G
                RGEWT
                        CIAYSQLRDQ
                                         С
                                           I
                                             V D
20
              G
          G
            N
            FHKRHEEGHMLNC
                                  T
                                    С
                                      F
                                       G
                                         Q
         DT
                DSETGTFYQIGDSWEKYVH
         D
          Q C
              Q
            GRGIGEWHCQPLQTYPS
                                         S S
                                            G P
                                                V
          Y
       ETPSQPNSHPIQWNAPQPSHISKYI
25
       VSIPPRNLGY
```

[SEQ ID NO: 1]

or natural variants thereof but not reactive towards fibronectin.

30

- An antibody reactive towards the polypeptide whose amino acid 15. sequence is shown in Figure 2 labelled pMSF1a between positions 19 and 660 or natural variants thereof but not reactive towards fibronectin.
- An antibody reactive towards an epitope present in the polypeptide 16. whose amino acid sequence is

```
35
    NLVATCLPVRASLPHRLN
                                LGTAVPSTGASKSK
    MLRGPGPG
                 LLLLAV
                            Q C
                   Q S
                       PVAVSQSKPGC
                                          YDN
              V
         Q
          Q M
                QP
                   LGNALVCT
                                 CYG
          WE
              R
                Т
                 Y
         Q
                       TGNT
                              YRVGDT
                                        YERP
         ΕE
              С
                F D
                   ΚΥ
            Т
                                 ANRCHE
              GAGRGRISC
                              T
40
          С
            I
                                I
                                            G
                            L
Y
                              E
                                  V
         WRRPHETGG
                         Y
                          М
                                С
                                   С
                                     L
                                       G
       Т
                          s
                              v
                                v
                                  GE
                         T
                                     T W
                       G
                                         Ε
                                          K
                                            Ρ
                                              Y
         ΕK
            С
              F
                D
                  Н
                   A A
     I A
                                 RNRCND
                   SG
                       R I
                          T
                            С
                              T
                                S
                                            Q D
                  G
           С
            L
              G
                Ε
            W S K K D N R G
V Q T T S S G S
                                  C I C T G N
D V R A A V
                              L
                                Q
T
     I G
         D T
                          N L
                                            G R
                          G P F
                                            ΥQ
     R H T S V Q T T S S G S
P P P Y G H C V T D S G
                                                Ρ
45
    ERHT
                          V
                            v
                              Y
                                s
                                  v
                                   GMQWLK
                                                Q
         T C L G N G
                       С
                                V
                                  Т
                                       Y
                                         G
     L C
                   V
                     S
                         Q
                          Ε
                            Т
                              Α
                                   Q
                                     Т
                                          G
                                            N
                       s
                         Ť
                            S
                                  E
                                     D
                           Т
                              NY
                                   Q
                                       QK
                                          Y
                                            S
                                              F
                                                С
         F
            YNDR
                   Т
                     D
                   s N
                       G A L C H F
                                  P
                                   E
                                     LYNNHN
     L V
         Q
          T
            R G
                G N
                       G T
                          TQNYDADQKF
                                            GF
                                                С
50
         RRDNMKWC
    SEG
    HEEICTTNEGVMYRIGDQWDKQHDMGHMMR
```

```
C T C V G N G R G E W T C I A Y S Q L R D Q C I V D D I T Y N V N D T F H K R H E E G H M L N C T C F G Q G R G R W K C D P V D Q C Q D S E T G T F Y Q I G D S W E K Y V H G V R Y Q C Y C Y G R G I G E W H C Q P L Q T Y P S S S G P V E V F I T E T P S Q P N S H P I Q W N A P Q P S H I S K Y I L R W R P V S I P P R N L G Y
```

5

20

[SEQ ID NO: 1]

or natural variants thereof but which epitope is not present in fibronectin.

- 17. An antibody reactive towards an epitope present in the polypeptide whose amino acid sequence is shown in Figure 2 labelled pMSF1α between positions 19 and 660 or natural variants thereof but which epitope is not present in fibronectin.
- 18. An antibody according to any one of Claims 14 to 17, reactive towards a molecule comprising any one of the peptides ISKYILRWRPVSIPPRNLGY [SEQ ID NO: 3] or QQWERTYLGNALVCTCYGGSR [SEQ ID NO: 4] or EPCVLPF-TYNDRTDSTTSNYEQDQ [SEQ ID NO: 14] or CTDHTVLVQTRGGNSNGA-LCH [SEQ ID NO: 15] or VGNGRGEWTCIAYSQLRDQCI [SEQ ID NO: 7].

19. An antibody reactive towards fibronectin but not reactive towards the polypeptide whose amino acid sequence is

```
MLRGPGPGLLLLAVQCLGTAVPSTGASKSK
    RQAQQMVQPQSPVAVSQSKPGCYDNGKHYQ
25
    INQQWERTYLGNALVCTCYGGSRG
                                               F N
     EAEETCFDKYT
                         G N T
                              Y
                                R V
                                    G D
                                IANRCHE
                             СТ
                                               G
            IGAGRGRIS
                                             G
           RRPHETGGYMLECVCLGNG
                                             KGEW
     IAEKCFDHAAGTSYVVGETWEKP
30
          \texttt{T} \texttt{C} \texttt{L} \texttt{G} \texttt{E} \texttt{G} \texttt{S} \texttt{G} \texttt{R} \texttt{I} \texttt{T} \texttt{C} \texttt{T} \texttt{S} \texttt{R} \texttt{N} \texttt{R} \texttt{C} \texttt{N} \texttt{D} \texttt{Q} \texttt{D} \texttt{T} 
     IGDTWSKKDNRGNLLQC
                                    ICTGN
     RHTSVQTT
                    SSGSGPF
                                 Т
                                  D
                                    VRAA
                                           V
                         G
                             V Y
                                 s v
                                    GMQWL
                  ٧
                    Т
                      D S
                           V
                                             K
             G H C
35
              G N G V S C Q E T A
                                 V
                                  T
                                    QT
                                        Y
                                         G
           C
       С
         Т
             L
                           TSNY
       PF
           Т
             YNDRTDST
                                  EQDQK
                                        YNNH
         QTRGGNSNGALCHF
                                  PF
                                      L
                         Т
                                 Y
                                  D A
                                      D
                                        QK
    SEGRRDNMKWCG
                           Т
                             Q
                               Ν
                         Y
                             Ι
                                      D
                                        K
         I C
              Т
                NEG
                      v
                        М
                           R
                               G
                                 D
                                   Q
                                    W
                                           Н
                                             D
                                               М
             Т
              GRGEWT
40
       С
         V G N
                         С
                           IAY
                                 S
                                  Q
                                    LRDQ
                                           С
                                             Ι
                                               V
      VNDTFHKRHEEGHMLN
                                    Т
                                      С
                                        F
                                           Q
      PVDQCQDSETGTFYQIGDSWE
                                           K
                                             Y
                                               V
                                                 H G
           YGRGIG.EWHCQPLQTYPS
                                           S
                                             S
                                               G
                                                 Ρ
                                                        F
       ΥC
                         IQWNAPQPSHISK
                                                 YILRW
      TETP
             SQPNSHP
45
        VSIPPRNL
```

NLVATCLPVRASLPHRLN

[SEQ ID NO: 1]

or natural variants thereof.

20. An antibody reactive towards fibronectin but not reactive towards the polypeptide whose amino acid sequence is shown in Figure 2 labelled pMSF1α between positions 19 and 660 or natural variants thereof.

21. An antibody reactive towards an epitope present in fibronectin but not present in the polypeptide whose amino acid sequence is

```
NLVATCLPVRASLPHRLN
10
   MLRGPGPGLLLLAV
                          QCLGTAVPSTGASKSK
   RQAQQMVQPQSPVAV
                            S
                             QSKPGC
                                       Y
                                         D
                                          N
                                            G
                          V
                            C
                             T
                               C
                Y
                  L
                   G N
                       A
                        L
                                Y
                                  G G S
                                       R G
                                          F
                                            N
      QQW
           ERT
                        N T
                            Y R
                               V
                                GDT
                                     Y
                   Y
                     Т
                       G
               F
                DK
          E
           T
             С
15
                GRGR
                       Ι
                         S
                          СТ
                             I
                               ANRCHEG
          C
           I
             G
              Α
        Т
                  T G G Y M L E C
                               V
        WRRPHE
                                CLGNGK
               DHAAGT
                         S Y
                            V
                             v
                               GET
                                    W
                                     Ε
                                       ΚP
           C F
        ΕK
                         T
                            T
          CLGEGSGR
                       I
                          С
                             S
                               R N R
                                    С
                                     N
                                       D
        T
                        N L
                            L
                             Q
                                I C
                                    Т
                                            G
                     R G
                               C
                                     G
                                       N
                                         G
          Т
           W S
               KKDN
       G
                       SGPF
                             T
                               DVRAA
20
        Т
          S
           V Q
               T
                T
                  S
                   S
                     G
                                       ٧
       Н
           G H C V T D
                        VVYSVGMQWLKT
                     SG
        Ρ
          Y
      ₽
                  V S C Q E T A V T Q T Y
                                     G G N
                                          S N
           LGNG
        Т
          С
           YNDRTDST
                         T S
                            N
                             Y
                               E
                                 Q D
                                    QK
                                       Y
                                         S
                                          F
        F
          T
              G N S
                     G
                       Α
                         L
                          С
                            Н
                             F
                               ₽
                                 F
                                  L
                                    Y
                                     N
                                       N
                                         Н
           R G
                   N
       V
        QΤ
                               D
                                 A D
                         T
                             Y
                                          F
25
       G
        R R
            D
             N
               М
                K
                  W
                    С
                     G
                       T
                          Q
                            Ν
                                    Q
                                     K
                                       F
                                         G
                                            C
                     M Y
                         R I
                    V
                            G
                             D
                               QWDK
                                     QH
                                         D M
                  G
             TNE
       E
        I C
            Τ
                  EWTC
        V G
           NGRG
                         I A
                            Y
                             S
                               QLRD
                                     Q
                                       С
                                          V
       С
           FHKRHEEGHMLNCTCFGQGRG
    D P V D Q C Q D S E T G T F
                          Y
                            OIGDSWEK
                               QTYP
                   EWHC
                                         S
                                          G
                                            Ρ
                                              V
30
          YGRGIG
                          QPL
                                     S
                                       S
       Y
        С
                  SHPIQWNAPQPSHISKYILRW
          Ρ
            S
             QΡ
                Ν
    RPVSIPPRNL
```

[SEQ ID NO: 1]

or natural variants thereof.

35

22. An antibody reactive towards an epitope present in fibronectin but not present in the polypeptide whose amino acid sequence is shown in Figure 2 labelled pMSF1 α between positions 19 and 660 or natural variants thereof.

40

23. An antibody according to any one of Claims 19 to 22 reactive towards a molecule comprising any one of the peptides

QQWERTYLGNVLVCTCYGGSR [SEQ ID NO: 8] OF EPCVLPFTYNGRTFYSCTTEGR-QDGHLWCSTTSNYEQDQ [SEQ ID NO: 9] OF CTDHTVLVQTQGGNSNGALCH [SEQ ID NO: 10] OF VGNGRGEWTCYAYSQLRDQCI [SEQ ID NO: 11] OF ISKYILRWRPKN-SVGRWKEA [SEQ ID NO: 12] OF peptides derived from position 648 in fibronectin as shown in Figure 2.

- 24. An antibody according to any one of Claims 14 to 24 which is a monoclonal antibody.
- 25. A method of making an antibody which is reactive towards the polypeptide whose amino acid sequence is

10

```
NLVATCLPVRASLPHRLN
                       L A V
   MLRGP
            G
              Ρ
                G L
                   L L
                            Q
                              CLGTAVPSTGASKSK
                         V A
                            v s
                       Ρ
                                QSKPGCYD
                                              N
                                                G
                                                 K
                   Q S
    RQAQ
          Q M
              V
                QP
                         ALVC
                                Т
                                 С
                                   YGGSRG
                  Y
                   L G
                       N
            Ε
              R
                Т
       QQ
          W
15
                          NTYRV
     Ε
       A
         Ε
          Ε
            T
              С
                F
                  D
                   K
                     Y
                       T
                         G
                                   GDTY
                       R
                         Ι
                           S
                            С
                              T
                                Ι
                                 ANRCHEG
         T
           C
            I
              G
                A
                  G
                   R
                     G
                         YMLECV
                                   CLGNGK
           R R
              Р
                H E
                   T
                     GG
                D H A A G T S Y V V G E T W E K P
         ΕK
            C F
            LGEGSGRI
                           TCTSRNRCN
                                          D
                                              D
     D C
         T
          С
20
                         G
                           N
                            L
                              L
                                         G
       G
         D
          Т
            WSK
                  KDNR
                                Q
                                  С
                                   Ι
                                     С
                                   v
       Η
           s
            ٧
              0
                Т
                  Т
                    S
                     S
                       G
                         S
                           G
                             Ρ
                              F
                                  D
                                     R
                                       A A
                                          V
                                            Y
                         G
                           V
                             V
                              Y
                                S
                                  v
       P
            G
              Н
                С
                  V
                    T
                     D
                       S
                                   GMQW
                                          L
                                            K
                   ٧
                     S
                       С
                           E T A
                                V
                                  T
                                     Т
                                       Y
                                         GG
                  G
                         Q
                                   Q
       С
         Т
           С
                N
            L
              G
                         T
                             S N Y
     L P
         F
           Т
            Y
              N
                D
                  RTDS
                           T
                                  E
                                   Q
                                     D
                                       QKY
25
                  NSNG
                         Α
                           LCHF
                                  P F
                                     L
                                       Y
                                         NNHN
       V
         Q
           TRG
                G
                         T
                           T
                             QNYDADQKF
                                            G
                                              F
                                                С
     EGRRDNMKW
                     C G
              TNEGVMYRIGDQWDK
         I C T
                                         Q
                                          H D M G
       CVGNGRGEWT
                         С
                           ΙA
                              Y
                                S
                                   L R
                                       D
                                          С
                                            Ι
                                              V
                                                D
     Ţ
                                  Q
                                         Q
     ٧
       NDTFHKRHE
                       Ε
                         G
                           Н
                            M L
                                     С
                                       F
                                            G
                                N
                                         G
                                           Q
                                              R
                                                G
30
                                            Y
                                              V
                                                   V
      ₽
       v
         D
           Q
            С
              Q
                D
                  S
                    Ε
                     T
                       G
                         T
                           F
                             Υ
                                Ι
                                  G
                                    D
                                     S
                                       W
                                         Ε
                                           K
                                                Н
                                                  G
                       WHC
                                L
                                   T
                                     Y
                                       Ρ
                                         S
                                          S
                                                 V
                  Ι
                    G
                     E
                             Q
                                            S
                                              G
                                                Ρ
                                                   Ε
         С
           Y
             G
              R
                G
                              Ρ
                                  Q
                         IQWNAPQPSHISKY
                  N S H P
      Т
         Т
           Ρ
             S
              Q
                Ρ
       E
       v s
           I P
              PRNL
```

[SEQ ID NO: 1]

- or a natural variant thereof and which is not reactive with fibronectin, the method comprising the steps of, where appropriate, immunising an animal with a peptide which distinguishes MSF from fibronectin and selecting an antibody which binds MSF but does not substantially bind fibronectin.
- 40 26. A method of making an antibody which is reactive towards fibronectin and which is not reactive towards the polypeptide whose amino acid sequence is

```
NLVATCLPVRASLPHRLN
                          L
                               V
                                 Q
                                   С
                                     L
                                       G
                                         T'A V P S
      LRGP
              G
                ΡG
                    L
                      L
                        L
                             A
                             v
                                 ν
                                   S
                                     Q S
                                         КР
                                             G
                                               С
                                                 Y
                                                   D
                                                     N
                                                        G
                         s
                               Α
                ٧
                  Q
                    Ρ
                       Q
                           Ρ
                         G N A
                                 V C
                                         Y
              Ε
                R
                  T
                    Y
                      L
                               \mathbf{L}
                                     T
                                       C
                                           G
                                             G
            W
                                     R \ V
                             GNTY
                    DKY
                           T
                                         G D
                                             T
          E
            Ε
              Т
                C F
                                     I
                                       ANRC
                GAGRGR
                             I
                               SCT
                                               H E G
                                                     G
                                     C
                                       V
                                         С
                                             G
              RPHET
                         GG
                             Y M L
                                   Ε
                                           L
                                               N
                                                 G
                                                   K
          W R
                                             W
                                                      Y
              С
                F
                  DH
                      Α
                         Α
                           G
                             Т
                               S
                                 Y
                                   V
                                     V
                                       G
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          E
                               Т
                                   Т
                                           R C N
                                                 D
                                                     D
                G
                   Ε
                     G
                       S
                         G
                           R
                             I
                                 С
                                     S
                                       R
                                         N
                                                   Q
                             G
                               N
                                 L
                                   L
                                     Q
                                       С
                                         ΙC
                                             T
                                               G
                                                 NG
                                                     R
                           R
10
              W
                   К
                     K
                       D
                         N
                S
              v
                   Т
                     \mathbf{T}
                       s
                         S
                           G
                             S
                               G
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                                           R A A
          T
            S
                Q
                                                     T
                               V
                                 V
                                   Y
                                     S
                                       V G M
                                             QWLK
          P
              G
                Н
                   С
                     v
                       Т
                        D
                           S
                             G
        P
            Y
                             Q
T
                       v s
                           С
                               ETAV
                                       T
                                             Y
                                               G
                                                 G
                                                   N
                                                     S
                                         Q T
          T
            С
              L
                G
                  ΝG
          F
                               T
L
              YNDRT
                         D S
                                 s
                                   N
                                     Υ
                                        E
                                         Q
                                           D
                                              Q
                                               K
                                                      F
        Ρ
            T
                                 С
                                   Н
                                     F
                                        Ρ
                                         F
                                              Y
                                                 N
                                                   Н
15
      L
               R
                G
                   G N
                       S
                         N
                           G
                             Α
                                            L
                                               N
        V
          0
            T
                             T
                               T
                                 Q
                                     Y
                                        D
                                         Α
                                           D
                                                 F
               D
                     К
                       W
                         С
                           G
                                   N
                                              QK
                                                   G
                                                      F
          R
            R
                N
                   М
                         V
                           М
                             Y
                               \mathbf{R}
                                 I
                                   G D
                                       QWD
                                              K
            С
               T
                 Т
                   N
                     E
                       G
                                               0
                                                 Н
                                                   D
          Ι
                                   Y S
                                       QLRDQ
          v
                     GEW
                           T
                             С
                               IA
                                                 С
            G
               N
                G
                   R
                               HMLNC
                HKRHEEG
                                         Т
                                             F
                                                 Q
                                                      R G
                                           С
                                                    G
               F
                                               G
                               F Y
                                   QIGDS
                                             W
                                                Ε
                                                  K
20
              C Q
                   D S
                       E
                         T
                           G T
          D Q
        v
        Y
          С
            Y
               G
                R
                   G
                     Ι
                       G
                         E
                           W H
                               С
                                 Q
                                   P
                                      L
                                        Q
                                         ·T
                                            Y
                                              Ρ
                                                S
                                                  S
                                                    S
                                                      G
                                                        P
                                                          V
                             IQWNAPQPSH
                           Ρ
                                                 I S
                                                      K
                                                        Y
             P
               S
                 Q
                   Ρ
                     N
                       S
                         H
            IPP
                       L
                           Y
                   R N
                         G
          S
```

[SEQ ID NO: 1]

- or a natural variant thereof, the method comprising the steps of, where appropriate, immunising an animal with a peptide which distinguishes fibronectin from MSF and selecting an antibody which binds fibronectin but does not substantially bind MSF.
- 27. A molecule which is capable of, following immunisation of an animal if appropriate, giving rise to antibodies which are reactive towards the polypeptide whose sequence is

```
CLPVRASLPHRLN
                                LA
                                     V
                    P
                             L
                                       QCLGTAVPS
                      G
                        L
                           L
35
                             S P
                                  VAVS
                                            QSKPGC
                    v
                        Ρ
                           Q
                 M
                      Q
                             GNALVCT
                                              CYGGSR
                                                             G
                 E
                   R
                           L
                    C F D K Y T
                                  G N T
                                          Y
                                            R V
                                                 GDT
                                                        Y
                                                          E
                                                             R
          AEET
                                  Ĭ
Y
          С
            Т
               С
                 I
                    G
                      Α
                        G
                           R
                             G
                                R
                                     S
                                       С
                                            Ι
                                              A N
                                                   R
                                                      С
                                                        Н
                                                          Ε
                                                             G
                                       L
                                          E
                                              ٧
                                            С
                                                   L G
                                                        N
                                                          G
                                                             K
                                                               G
                           T
                             G
                                G
                                     M
                                                 C
          \mathbf{T}
                 R
                    Ρ
                      Н
                        Ε
                                       Y V V G E T W
C T S R N R C
                           Α
                                  T
                                     s
                                                        E
40
                 С
                    F
                      D
                        Н
                             A
                                G
                                                          K
               K
                                   I
                                     T
          С
                 L
                    G
                      E
                        G
                           S
                             G
                               R
                                                        N
                                                          D
               С
                                       ŗ
                                  G N
                    S K
                           D N
          G
               Т
                 W
                        K
                                R
                                          L
                                            Q
                                              С
                                                 Ι
                                                   С
                                                      T
                                                        G
                                                          N
                                                             G
                        T
V
                           S
T
V
                             S
                                   S
                                     G
V
                                       P
V
                                G
S
                                          F
Y
                                             T
S
                 V
                    Q
                      T
                                               D
                                                 V
                                                    R
                                                      Α
                                                        Α
          Н
            Т
               S
                    Н
                      С
                                               V
                                                 G
                                                      Q
                                                        W
                                                          L
                                                             K
                 G
                                                   М
                                       T A V
S N Y
C H F
                                  Q
T
A
45
          C
             Т
               С
                    G
                        G
                              S
                                С
                                     Ε
                                               T
                                                 Q
                                                   T
                                                      Y
                                                        GG
                                                             N
                 L
                      N
                                S
G
                                     T
L
                                                 Q
F
          ₽
             F
               T
                 Y
                    Ν
                      D
                        R
                           T
                             D
                                            ΥE
                                                   D
                                                      QK
                                                           Y
                                                             S
                                               P
                                                      Ŷ
                                                   L
                                                          N H
                                                                  Y
             Q
               Т
                 R
                    G
                      Ģ
                        N
                           S
                             N
                                                        N
                                  T
Y
                                       Q
I
A
                                G
                                            Y
               R D
                    NMKW
                              С
                                     Т
                                          N
                                               D
                                                 Α
                                                   D
                                                      Q
                                                        K
                                                           F
                                                             G
                                                                F
                                                                  C
                                                                     P
          G
            R
          E
             I C
                 T
                    T
                      N E
                           G
                              v
                                М
                                     R
                                          G
                                            Đ
                                               Q
                                                 W
                                                    D
                                                      K.
                                                        Q
                                                          Н
                           Ē
                                   С
                                     I
                                          Y
                                             S
50
                              W
                                Т
                                               Q
                                                 L
                                                      D
                                                                V
          C
             V
               G
                 N
                    G
                      R G
                                                    R
                                                           С
                                                             Ι
          N
                 F
                    Н
                      K
                         R
                           Н
                              E
                                E
                                   G
                                     Н
                                        М
                                          L
                                            N
                                              C
                                                 T
                                                    С
                                                      F
                                                        G
                                                           Q
                                                             G
            D
               T
          V D Q C Q D S E T G T F Y Q I G D S W E K Y V H G V Y C Y G R G I G E W H C Q P L Q T Y P S S S G P V E
```

or natural variants thereof but not reactive towards fibronectin.

28. A molecule which is capable of, following immunisation of an animal if appropriate, giving rise to antibodies which are reactive towards fibronectin but not reactive towards the polypeptide whose sequence is

```
NLVATCLPVRASLPHRLN
   MLRGPGPGLLLLA
10
                          Q
                           CLGTAVPSTGASKSK
                  Q S P V A V S Q S K P G C Y D N G K H Y Q
      AQQMVQP
      QQWERTYLGNALVCTCYGGSRG
              FDKYTGNT
                            YRVGDTYER
      AEETC
         CIGAGRGRI
                        s c
                            Т
        Т
                             Ι
                               ANRC
                                     н Е
                                         G
15
      TWRRPHETG
                     G
                       Y
                        M L
                            E
                             С
                               V
                                 С
                                  L
                                    G
                                     N
              рнаа
                       Т
                        S
                            v
                             V
                               G E
                                  T
      Α
        EKC
             F
                     G
                          Y
                                    W
                                     E
                     RIT
                          С
                            T
                             S
                               R N
                                  R
                                    C N
                                       D
                                         Q
                                          D
          C
             G
              E
                G
                  S
                   G
                     R G
                        N
                          L
                            L
                             Q
                               С
                                 Ι
                                  C
                                    T
                                     G
                                       N
              KKDN
           W
             S
                             \mathbf{T}
                               DVRAA
                   S
                     G
                      S
                        G P
                            F
        Т
          S
           V
             Q
               T
                T
                  S
      Н
20
              CVTD
                     S
                       G V
                          V
                            YSVGM
                                    QWLK
      Р
          Y
           G H
        Ρ
              NG
                  v s
                     С
                       QETAVT
                                 QT
                                    Y G
                                       G
                                          SNG
      C
        T C
           L
             G
                     STTSNYEQDQK
                                       Y
                                         S
           ΥN
              D R
                  T D
              GNSN
                     GAL
                            HFPFLYNN
                                         Н
                          С
        QTRG
     EGRRDNMKWC
                     G
                       T
                        Т
                          Q
                            N
                             Y
                               D A
                                  D
                                    Q
                                     K
                                       F
                                         G
                                          F
                                            С
25
                  G
                   V
                     Μ
                       Y
                         R
                          Ι
                            G
                             D
                               Q
                                 W
                                  D K
                                     Q
                                         D
                                          Μ
      EICTT
               N
                E
                                       Η
                     Т
                       С
                            Y
                             S
                                       С
        V
           N
             G
               R
                G
                  Ε
                   W
                         Ι
                          Α
                               Q
                                 L
                                  R
                                    D
                                     Q
                                         Ι
                                          V
                                            D
          G
                            L N
                               С
               KRHE
                     E G
                        н м
                                 Т
                                  С
                                    F
                                     G
        D
          T
           F
             H
                                       Q
                                         G
                                          R G
                SET
                             ΙG
                     GT
                        F Y Q
                                 D S
                                    WE
                                       K
       V D
          Q
           С
             Q
               D
                     WHCQPLQTYPSSSGP
        С
          Y G
               G
                I
                  GΕ
      Y
             R
                       IQWNAPQPSHISKYILRW
30
               PNSHP
     T E
        T
         P S Q
     PVSIPPRNLGY
```

or natural variants thereof.

5

40

29. A molecule according to Claim 27 which is a peptide comprising any one of the sequences

ISKYILRWRPVSIPPRNLGY [SEQ ID NO: 3]; or

QQWERTYLGNALVCTCYGGSR [SEQ ID NO: 4]; or

PCVLPFTYNDRTDSTTSNYEQDQ [SEQ ID NO: 5]; or

TDHTVLVQTRGGNSNGALCH; or [SEQ ID NO: 6]; or

VGNGRGEWTCIAYSQLRDQCI [SEQ ID NO: 7]

which are found in MSF.

30. A molecule according to Claim 28, which is a peptide comprising any one of the sequences

QQWERTYLGNVLVCTCYGGSR [SEQ ID NO: 8] or

EPCVLPFTYNGRTFYSCTTEGRQDGHLWCSTTSNYEQDQ [SEQ ID NO: 9] or

CTDHTVLVQTQGGNSNGALCH [SEQ ID NO: 10] or

VGNGRGEWTCYAYSQLRDQCI [SEQ ID NO: 11] or

ISKYILRWRPKNSVGRWKEA [SEQ ID NO: 12] or

peptides derived from position 648 onwards in fibronectin as shown in Figure 2.

31. A polynucleotide which is capable of distinguishing a polynucleotide which encodes the polypeptide whose sequence is

```
NLVATCLPVRASLPHRLN
                           LGTAVPSTGASKSK
15
   MLRGPGPG
               L L
                  L
                    L
                     A V
                        Q
                          С
                        V S
                           QSKPGCYDNGKHY
                     V A
                  S P
   RQAQQM
            V
             Q
               P
                Q
                  GNALVCTCYGGSRGFN
      QQWERT
               ΥL
            CFDKYTGNTYRVGDTYERPKDS
       EET
         CIGAGRGRISCTIANRCHEGG
20
    DTWRRPHETGGYM
                        L
                          E
                           С
                              С
                                L
                                    G
                        Y
                                       Y
                     Т
                          V
                           V
                             G E
                                T W
                                   EKP
       Ε
         КC
            F
              DHAA
                    G
                       S
                  G R I T
                        CTSRNRCNDQDT
              E
                 S
           L
            G
               G
           WSKKDNRGNLL
                           Q
                             С
                              ICTGNGRGEWKC
       D
         Т
      G
          V Q T T S S G S G P F T D V R A A V Y Q P
    RHTS
    PPPYGHCVTDSGV
                        V
                          Y S V
                              GMQW
                                    LKT
25
      CTCLGNGV
                  s c
                     Q
                       Ε
                        Т
                          Α
                           V
                             \mathbf{T}
                              Q
                                T
                                 Y
                                   G
                                    G N
                                       S N
                    S
                     T
                       T
                        s
                          N
                           ΥE
                                    Y S
                              QDQK
               R T
                  D
     L P
       F
         T.Y N D
             GNSN
                    GALC
                          H F
                             P
                                L
      V
         TRG
        Q
                    G T T Q N Y D A D Q K F
      GRRDNMKWC
                                      G
       ICTTNEGVMYRĪ
                          GDQW
                                DKQH
                                      DMG
30
     TCVGNGRGEW
                    Т
                     С
                       I
                        Α
                          Y
                           S
                              L R
                                 D
                                    С
                             Q
           F H K R H E E G H M L N
                             С
                              T C
                                 F G.O
      NDT
                                      G.R.G
      V D Q C Q D S E T G T F
                        YQIGDSWEKYVHG
         YGRGIGEWHCQPLQTYPSSSGPV
      ΥC
   I T E T P S Q P N S H P I Q W N A P Q P S H I S K Y I L R W
35
   RPVSIPPRNLGY
```

[SEQ ID NO: 1]

or a natural variant thereof and a polynucleotide which encodes fibronectin.

32. A polynucleotide which is capable of hybridising to a polynucleotide which encodes fibronectin but not a polynucleotide which encodes the polypeptide whose sequence is

```
NLVATCLPVRASLPHRLN
5
    M L R G P G P G L L L L A V Q C L G T A V P S T G A S K S K
       Q A Q Q M V Q P Q S P V A V S Q S K P G C Y D N
           Q W E R T Y L G N A L V C T C Y G G S R G F E E T C F D K Y T G N T Y R V G D T Y E R P
                                       YRVGDTYERPK
                                       T I
E C
                         RGRISC
                IGAG
                                            ANRC
                                                     H E
                                                          GG
10
             RRPHETGG
                                Y M L
                                            V C
                                                 L
                                                   G
                                                     N G
                                                          K
                                                            G
                    DHAAG
                                Т
                                  S
                                     Y
                                       v v
                                            G
                                              E
                                                 T
                                                   W
                                                     Ε
                                                            Y
           ΕK
                C F
                                                       K
                                                          Ρ
                LGEGSGRITCTSRNR
WSKKDNRGNLLQCIC
                                                   CND
           T
              С
                L G
                                                          Q D
                                                   T
                                                     G N
           TSVQTTSSGSG
                                     P F
                                            D V
                                                 R A
                                          T
                                                     Α
                                                       V
                                                          Y
                                G
Q
T
                              S
C
                                          s
v
15
           P
              YGH
                     C
                       V
                         T D
V S
                                   v
                                     V
                                        Y
                                            v
                                              G
                                                 M
T
                                                   QW
                                                        L
                                                          ĸ
                                                               Q
                                   E T
                                       Α
                                            T
            T
              С
                  G
                     N
                       G
                                              Q
                                                     G
                                                        G
                                                          N
                     DRTDS
                                   Т
                                     s
                                       N Y E
                                                 D
                                                   Q
                                                     ΚY
                                              Q
                                                          S
           F
              Т
                YN
           Q T R G G N S N G A L C H F P F L Y N N H N Y T D C T
R R D N M K W C G T T Q N Y D A D Q K F G F C P M A A
I C T T N E G V M Y R I G D Q W D K Q H D M G H M M R
20
                                            Q L R
C T C
           VGNGRGEWT
                                С
                                   ΙA
                                       Y S
                                                   DQC
                                                          I
                                                            V
                  HKRHEEGHMLN
                                                   F
                                                     G
           D
                F
                                                          G
                     DSETGTFYQIGDSW
                                                     ΕK
         V D
                С
                                                          Y
                                                            V
                                                              Н
                  Q
                     G I G E W H C Q P L Q T Y P S S S G P V P N S H P I Q W N A P Q P S H I S K Y I
           С
              Y
                G R
         Y
25
           Т
              Ъ
                S Q
         VSIPPRNLGY
```

[SEQ ID NO: 1]

or a natural variant thereof.

NLVATCLPVRASLPHRLN

30 33. A polynucleotide which is capable of hybridising to a polynucleotide which encodes the polypeptide whose sequence is

MLRGPGPGLLLLAV Q LGTAVPSTGASKSK P Q S Q M Q Ρ Q S VAV S KPG 35 NALVC Т C Y R T Y L G GG SRG Т С F D K Y T G N T Y R V G D T Y E R P K D GRGRISCT IANRCHE G A DTW RRPHETGGYMLECVC LGNGK ΕK CFDHAAGT s y vV GΕ T W ΕK 40 SGRI Т С Т S С L G Ε G R N R C N D T L G D D N R N L I C T W S K К G Q С T G N G S G D S S G G V P F V Y Ť s v T D V RAAV Т Q T s Y G H С V T V GMQWLK Q E T A V T T S N Y A L C H F LCT CLGNGVSC Т Т Y G N G S Q E P Q 45 P Т Y Т D s D Q K F N D R Ğ N Т R Ģ G N S L Y D A R K W С G T T Q N D Q KF D N М Y R I G D Q W D C I A Y S Q L R I C TTNEGVM KQHD GNGRGEWT D Q v С Ι V 50 NDTFHKRHEEGHMLN С T С F G Q G R G I L $\mathsf{D} \ \mathsf{Q} \ \mathsf{C} \ \mathsf{Q} \ \mathsf{D} \ \mathsf{S} \ \mathsf{E}$ T G Т F Y G D S W £ G Q v R QP GE WHC Y Ι Q T Р S S V С Y G R G S G Ρ QPNSHP IQWNAPQPSHISKYI P S Ε Т VSIPPRNL

[SEQ ID NO: 1]

or a natural variant thereof but not to a polynucleotide which encodes fibronectin.

- 5 34. A polynucleotide according to any one of Claims 31 to 33, wherein the polynucleotide is an oligonucleotide.
 - 35. A polynucleotide according to any one of Claims 31 to 34, wherein the polynucleotide which encodes fibronectin or the polynucleotide which encodes the polypeptide as said or a natural variant thereof is a mRNA or a cDNA.

10

15

36. A method of diagnosing cancer the method comprising detecting in a sample from the person to be diagnosed the presence of a polypeptide whose sequence is

```
NLVATCLPVRASLPHRLN
                       V
                        QCLGTAV
                                   PSTGASKSK
   MLRGPGPGLLL
                    LΑ
                            QSKPG
                                     Υ
                                      D N
                     V A
                        V S
                                   C
    OAOQMV
              Q
               Ρ
                 Q S
                    P
                  GNALVCTCY
               Y L
                                G G S
   INQQWERT
                               GDTY
20
       EET
            С
             F
               D K Y
                    Т
                     GNTYRV
                                     ERPK
               GRGRISCTIANRCH
                                     Ε
                                      GG
         С
            G A
   GDTWRRPHETGGYMLEC
                             v
                               С
                                L
                                  G
                                   N
                                        Y
                                Т
                                      Ρ
              DHAA
                    G
                     Т
                       S
                         Y
                          V
                            V
                             G E
                                  WE
                                     K
      Α
       EKC
            F
                                      QDT
                     I T
                        CTSRNRCND
                  G
           L
            G
              Ε
               G
                 S
                    R
25
             KKD
                  NRGNLLQ
                             С
                               ΙC
                                  TGNGRG
      G
       D
         Т
          W
            S
                             DVRAA
                                     VYQ
                  S
                    GSGPFT
    R H
       Т
         S
          V Q
              T
               T S
               VTDSGVVYS
                             VGMQWL
                                      K
                                        T
          GHC
                     Q
T
                  s
                         Т
                            V
                             Т
                                T
                                   G
                                     G
                                      N
          LGNG
                V
                    С
                       E
                          Α
                               Q
      С
       T C
                                     Y
           Y
            N
              D
               R
                 T
                  D S
                       T
                         s
                          Ν
                            Y
                             E
                               Q
                                D
                                  QK
                                      S
                                        F
      Ρ
         T
                                  YNN
              G N S
                         С
                            F
                             Ρ
                               F
30
                  NG
                     Α
                       L
                          Н
      v
        Q
         T
          R
            G
            N M
                     T
                       Т
                         QN
                            Y
                             DADQK
                                     F
                                        F
         R D
               K W
                  C G
      G
       R
                    MYRI
                                      D
                                        M G
                          GDQW
                                DKQH
                  V
         С
            Т
              NEG
         GNGRGEWT
                     C
                       ΙA
                          Y
                            S
                             Q
                               L
                                R
                                   Q
                                     С
      С
       V
                                        R G
      NDTFHKRHE
                       Н
                         М
                          L
                            N
                             С
                               Т
                                С
                                  F
                                   G
                                      G
                          QIGD
                                SWEKYVH
                         Y
35
                  Т
                    G
                     Т
                       F
      V
           С
            Q
              D
               SE
                  EWHCQPLQT
                                     SSGP
                                           V
                                ΥP
      Y
        С
         YGRG
               ΙG
     TETPSQPNSHPIQWNAPQPSHISKYILRW
   RPVSIPPRNLGY
```

[SEQ ID NO: 1]

or a natural variant or fragment thereof using a reagent which can distinguish said polypeptide from fibronectin.

37. A method of determining susceptibility to cancer the method comprising detecting in a sample derived from the person to be tested the presence of a polypeptide whose sequence is

```
NLVATCLPVRASLPHRLN
            GLLLLAVQCLGTAVPSTGASKSK
          P
            Q P Q S P V A V S Q S K P G C Y D N G K H Y Q
      Q Q M V
      Q W E R T Y L G N A L V C T C Y G G S R G F N C E S K
    EĀĒETCFDKYTGNTYRVGDTYERPKDS
         IGAGRGRISCTIANRCHEGGQ
10
    DTWRRPHETGG
                  Y
                    MLE
                        С
                         V
                          С
                            LGNG
                                 K
                   SYVV
                            TWEK
      EKCFDHAAG
                  Т
                         GE
                                 PΥ
      TCLGEGSGRI
                    TCTSRNRCNDQDT
         WSKKDNRGNLLQCICTGN
     G
      D T
        SVQTTSSGSGPFTDVRAAV
                                 Y Q P
15
    PPPYGHCVTDSGVVYSVGMQWLKT
                  QET
      TCLGNGVSC
                      AVT
                            Т
                              G
                                G
                                 N
     С
                  TT
                      NYEQDQKY
                                 S
         YNDRTDS
                     S
      F
         RGGNSNGALCHFPFLYNNHN
        T
       0
     GRRDNMKWCGTT
                     QNYDADQKF
20
      ICTTNEGVMYRIGDQWDKQHDMGH
     CVGNGRGEWTC
                    IAYSQLRDQC
                                 IVDD
    VNDTFHKRHEEG
                    H M L
                        N
                          Т
                            С
                             F
                              G
                                Q
                                 G
                                  R
                                    G R
                     Y
                              ΕK
                                 YVHG
                    F
                        IGDSW
       DQ
         С
           Q D S
              E
                T
                 G
                   Т
                       Q
25
               GEWHCQPLQTYP
                              SSSGPV
        Y
             I
     Y
       С
         G
           R
            G
                          QPSHISKYILRW
             NSHPIQWNAP
     Ε
       Т
        Ρ
         S Q P
     VSIPPRNLGY
```

[SEQ ID NO: 1]

or a natural variant or fragment thereof using a reagent which can distinguish said polypeptide from fibronectin.

38. A method of determining the likely outcome of a patient with cancer the method comprising detecting in a sample from the patient the presence of a polypeptide whose sequence is

```
NLVATCLPVRASLPHRLN
35
                         Q C L G T A V P S T G A S K S K
   MLRGPGPGLLLLAV
                  S P V A V S Q S K P G C G N A L V C T C Y G G S
         QMVQPQSPVAVS
                                     Y D
                                     R G
                                        F N
                Y L
         WERT
               D K Y T G N T Y R V G D T Y E R P K D
             С
              F
           IGAGRGRISCTIANRCHEG
40
        Т
         С
        WRRPHETGGYMLECVCLGNGKGEWT
     I A E K C F D H A A G T S Y V V G E T W E K P Y
           LGEGSGRI
                       TCTSRNRC
                                   N D
   V D C
        T C
                KDNRG
                       N
                         L
                           L
                            Q
                              С
                               I C
                                  Т
                                   G N
                                       G
                                        R
        D
         TWSK
                 SSGS
45
                       G P F
                            TDVRAAV
                                       Y
                Т
        T
         s
           V
             QT
           GHC
                VTDSG
                       V
                         VYSVGMQWLK
        Ρ
         Y
           LGNGVSCQETAVTQT
                                  Y
                                    GGN
                                        S N
        Т
      PFTYNDRTDS
                      Т
                        T
                         SNYEQD
                                  QK
                                       S
                      Α
                        L
                         CHF
                               FLYN
                                     ΝН
                                            T
      V
        Q
         Т
           RGG
               N
                 S
                   ·N G
                              ₽
                                        NY
                      T
                        TQNYDADQKF
                   CG
                                       G
                                        F C
50
           D
             N
              М
                K W
        ICTTNEGVMYRIGDQWDKQHDMGHMMR
```

```
CTCVGNGRGEWTCIAYSQLRDQCIVDDIT
 VNDTFHKRHEEGHMLNC
                         С
                        Т
                           F
                            G
                               G
                                R
                     Ι
                       G
                        D
                         S
                           W
                                V
                                  Н
                                   G
D P V D Q C Q D S E T G
                T
                 F
                    Q
                        TYPS
                             SSGP
                                   V E
                   QP
     YGRGIGEWHC
                     L
                       Q
                        QPSHISKYIL
                IQWNAP
     PSQPNSHP
        PRNLGY
     Ι
      Ρ
```

[SEQ ID NO: 1]

or a natural variant or fragment thereof using a reagent which can distinguish said polypeptide from fibronectin.

10

- 39. A method according to any one of Claims 36 to 38, wherein the reagent which can distinguish said polypeptide from fibronectin is an antibody according to any one of Claims 14 to 18.
- 15 40. A method of diagnosing cancer the method comprising detecting in a sample from the person to be diagnosed a polynucleotide encoding a polypeptide whose sequence is

```
NLVATCLPVRASLPHRLN
                               L
                                 G
                                  TAVPSTGASKSK
   MLRGPGPGLLLLA
                           Q
                                    PGC
                                         Y
                                           D N
                           V
                             S
                               Q
                                 S
                                  K
20
       A Q Q M V
               QΡ
                   Q
                     S
                      Ρ
                        V
                          Α
                     GNALVC
                               Т
                                 С
                                  YGGSRGF
            E R
               T
                 Y
                   L
               FDKY
                      Т
                        GNTYRVGDTY
                                         ERP
            T
              С
         E E
              GAGRGRISCT
                               IANRCHEGG
            I
       С
          С
         WRRPHETGGYMLEC
                                 V
                                  С
                                    L G
                                        N
                                         G
                           Y
C
                                           ₽
                                             Y
                          S
                             V
                               V
                                 G
                                  Ε
                                        E
25
            С
              FDHAAG
                        T
     IAEK
                          T
                               S
                                    R
                                      С
                                         D
                                           Q D
            LGEGSG
                        Ι
                             Т
                                 R
                                  N
                                        N
                      R
         T C
    V D C
                            L
                             L
                               Q
                                      Т
                                        G
                   D
                     N
                      R
                        G
                          N
                                 C
                                   Ι
                                    С
       G D
          Т
            W
              S
                К
                 K
                          G
                            Ρ
                             F
                               T
                                 D
                                   V R A
                   S
                     S
                      G
                        s
                Т
                 Т
       Н
          S
            V
              Q
                                   GMQWLKT
                                               Q
                        G
                          V
                            VY
                               s v
          Y
            G
              Н
               С
                 V
                   Т
                     D
                      S
         Ρ
                                      Y
                     s c
                        QETA
                               v
                                 Т
                                   Q
                                    T
                                        G
                                          G
30
         Т
          С
            L
              G
               N
                 G
                   V
                        T
A
                      S
                                          Y
                     D
                          \mathbf{T}
                            S
                             N
                               Y
                                 Ε
                                   Q
                                    D
                                      Q
                                        K
                                           S
              N D R
                   Т
            Y
          Т
                          L
                            C
                                   F
                                      Y
                             Н
                               F
                                 Р
                                    L
                                        N
                                         N H
                                             N
          T R
              G
                G
                 N
                   S
                     N
                        Т
                          T
                               Y D A D
                                      QK
                                         F
                                           G
                   W
                     С
                      G
                            QΝ
                 K
            D
              N
                M
                                               G
                      MYRI
                               DQWDKQHDM
            T T N E G
                     V
                             G
         I C
       Ε
         VGNGRGEWTC
                          I A Y S
                                 QLRDQ
                                          С
                                           I
35
     VNDTFHKRHEEGH
                            Μ
                             LN
                                 С
                                   Т
                                      F
                                        G
                                          Q
                                           G
                                             R
                                      WE
                                          K
              QDS
                                   D S
                   E
                     Т
                       G
                        T F
                            Y
                             Q
                               I
                                 G
     ₽V
         DQC
                     EWHC
                            QPLQTYP
                                        s
                                          S
                                           S
                                             G P
                G
                 Ι
                   G
         С
            G
                                             KYILRW
                        IQWNAPQP
                                      s H
                 N S H P
         T
           P
            S
              Q
                Ρ
       VSIPPRNLGY
40
```

[SEQ ID NO: 1]

or a natural variant thereof using a reagent which can distinguish said polynucleotide from a polynucleotide encoding fibronectin.

41. A method of determining susceptibility to cancer the method comprising detecting in a sample derived from the person to be tested the presence of a polynucleotide encoding a polypeptide whose sequence is

```
NLVATCLPVRASLPHRLN
        GPGPGLLLLAV
                            CLGTAVPSTGASKSK
                          Q
      R
              QPQSPVAV
                            SQSKPG
                                       Y
          QMV
                          v
                                  GGS
                                       R
                                        Ģ
                                          F N
                   G N A
                        L
                            С
                               С
                                 Υ
           ERT
                Y L
                          T
                            Y
                               V
                                GDT
                                     Y
                                       ERPKD
                             R
                D
                  K
                   Y
                     Т
                       G
                        N
             С
              F
                        S
                          С
                            TIANRCHE
                  R
                   G
                     R
                       Ι
             G
              Α
                G
                     GYMLEC
                               V
                                CLGNG
                                         KGEW
                  Т
                   G
10
          R
           R
             P
              Н
                Ε
                             V
                               GETWEKP
           CFDHAAGTSY
                            V
      Α
        Ε
          K
              EGSGRITCTSRNRCN
        Т
          С
           L G
        DTWSKKDNRGN
                          L
                            L
                                 I
                                  С
                                    Т
                                      G
                                       N
                                         G
                             Q
                               С
                                 V
                                    Α
                                         Y
                T
V
                          Ρ
                            F
                             {\tt T}
                               D
                                  R
                                     Α
                                       V
      Н
        Т
          S
           V Q T
                  S
                   S
                     G
                       S
                        G
                  T
                          v
                            Y
                             s
                     s
                       G
                        V
                               V
                                G M
                                     W
                                       L K
               С
                    D
                                    Q
15
       P
        P
          Y
           G H
           L G
                G
                  v
                   S
                     С
                       QE
                          T
                            Α
                             V
                               T
                                 Q
                                  Т
                                      G
                                       G
              N
       С
        T
          С
                                       Y
                          s
                            NY
                               E Q D
                        Т
                                    Q
                                     K
           Y N
                  T
                   D
                     S
                       T
       P
        F
          Т
               D R
               GNSNGALC
                            H F
                               P
                                 F
                                  L
                                    Y
                                      N
                                       N
                                         H N
           R
             G
       V
        Q
                     G T
M Y
        RRDNMKWC
                         Т
                          Q
                            N
                             Y
                               D A
                                  D
                                    Q
                                       F
                               QWDK
                                         D M
               N E
                         R
                          I
                            G
                             D
                                      QH
                  G
                    v
20
        I
          С
           T
             Т
                  E W
                     T C
                         I A
                            Y S
                               QLRD
                                     Q
                                       С
                                         ΙV
          G
           N
             G
               R
                G
                     ΕG
                        HMLNCTCF
                                      G Q
            FHKRHE
        D
          T
                            QIGDSWEK
                                         ΥV
          QCQDSET
                     G T
                        F
                          Y
                                            Н
        D
           GRGIGEWHCQPLQTYPSSSG
                                            P
                                                E
          PSQPNSHPIQWNAPQPSHISKY
                                              I
25
       E T
    RPVSIPPRNLG
```

[SEQ ID NO: 1]

or a natural variant thereof using a reagent which can distinguish said polynucleotide from a polynucleotide encoding fibronectin.

30

42. A method of determining the likely outcome of a patient with cancer the method comprising detecting in a sample from the patient the presence of a polynucleotide encoding a polypeptide whose sequence is

```
NLVATCLPVRASLPHRLN
                             GTAVPSTGASKSK
35
   MLRGP
           GPGLLL
                    L
                     Α
                       V
                        Q
                          С
                           L
                 QSP
                     VAVS
                           QSKPGCYDN
           MVQ
               Ρ
                  G N A L V C
              Т
               ΥL
                           Т
                             С
                              YGGSR
          E R
         W
                     GNTYRV
                              GDTY
               DKYT
         Ε
           Т
            С
             F
               GRGR
                     I S C T
                           IANRCHE
            G A
                     Y
                        L
                          E
                           С
               E T
                  G G
                       М
                             v
                               С
                                L
                                   N
                                      K
40
         RRPH
                        Y V
                     Т
                       s
                           v
                             G
                              Ε
                                Т
                                 WEKP
                 A A
                    G
            F
              D
               Н
               G S G R I T
                        С
                          TSRNRCNDQ
            G
              Ε
           WSKKDNRGNLL
                              ICTGNGRG
                           Q
                             С
         Т
                           Т
                             DVRAAV
                 SSGSGPF
                                      Y
        Т
         S
           V
            QT
               T
               VTDSG
                       V
                        V
                          Y
                           s
                             ٧
                               G M
                                  QWL
45
         YGHC
                 v
                  s
                    С
                     Q
                       Ε
                        T
                          Α
                           V
                             Т
                               Q
                                Т
                                  Y
                                   G
                                     G
                                      N
           L G N G
        T C
      C
                     T
                        SNYE
                                D
                                    Y
                  D S
                       T
                               Q
                                  QΚ
      P
        F
         Т
           Y N D
               R T
           RGGNSNGALCHF
                               F
                                L
                                  Y
                             P
         T
                        QNYDADQK
                                        F
                                    F G
         R
           DNMKW
                  C G
                     T
                       Т
      G
        R
           TTNEGVMYRIGDQWD
                                 KQHD
                                        M
                                           н м
50
         С
                                       V D
        VGNGRGEWT
                     С
                       IAYS
                             QLRDQ
                                     С
                                      I
                                           D
    NVNDTFHKRHEEGHMLNČTCFGQGRGRWKC
```

```
D P V D Q C Q D S E T G T F Y Q I G D S W E K Y V H G V R Y Q C Y C Y G R G I G E W H C Q P L Q T Y P S S S G P V E V F I T E T P S Q P N S H P I Q W N A P Q P S H I S K Y I L R W R P V S I P P R N L G Y
```

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10

15

[SEQ ID NO: 1]

or a natural variant thereof using a reagent which can distinguish said polynucleotide from a polynucleotide encoding fibronectin.

- 43. A method according to any one of Claims 40 to 42, wherein the reagent which can distinguish said polynucleotide from a polynucleotide encoding fibronectin is a polynucleotide according to Claim 31 or 33.
- 44. A method according to any one of Claims 36 to 43, wherein the cancer is breast cancer.
- 45. Use of a reagent which can distinguish the polypeptide whose sequence is

```
NLVATCLPVRASLPHRLN
                           CLGTAVPSTGASKSK
   MLRGPGPGLLLLA
                          Q
                 Q S P V A V S Q S K P G C Y D N G
   RQAQQMVQP
20
   INQQWERTYLGNALVCT
                              CYGGSRGF
        EETCFDKYTGNT
                           Y
                             R V
                                GDT
           IGAGRG
                     R
                      Ι
                        S
                          С
                           T
                             1
                              A N
                                  R C
                                     ΗE
                                        GG
        T C
                                  LGNGKGEW
                          L E C
                              V
                      Y
                                С
                  Т
                     G
                        М
        WRR
             Ρ
              H E
                   G
                 A A
                     G
                      Т
                        s
                          Y V
                             VGETWEKPY
25
           С
             F
              D H
        Ε
         K
           LGEGSGRIT
                          CTSRNRCNDQDT
         С
                             QCICTGNGR
      GDTWSKKDNRGNLL
             QTTSS
                        G
                          ₽
                            F
                             T
                               D
                                V
                                  R A
     RHTSV
                     G S
                      G
                        v
                          v
                            Y
                             s
                              V
                                G
                                  M Q
                                     WLK
              С
                v
                  Т
                   D
                     S
     P P P
         Y
           G
             Н
                  v
                             V
                                   Y
                                     GGN
30
                G
                   s
                     С
                      Q
                        Ε
                          T
                           Α
                              T
                                Q
                                  Т
        Т
          С
           L
             G
              'N
                             ΥE
                                        S
                  Т
                   D
                     S
                        Т
                          S N
                                Q D Q K
                                      Y
       Ρ
          Т
           Y
             N
              D R
                   NGALCHF
                               ₽
                                  LYN
                                        Н
                  S
                                F
                                       N
          Т
           R
             G
              G N
                          Q
I
                        Т
                            N
                             Υ
                               D
                                Α
                                  D
                                     K
       GRRDNMKW
                   С
                     G
                      T
                                   Q
                      Y
                            G
                             D
                                  D
                                        D
                                          М
                                            G
           Т
             Т
              ΝE
                  G
                   V
                     М
                        R
                              Q
                                W
                                   K
                                     Q
                                       Н
          С
                          A Y S
                               QLRD
                     Т
                      С
                        Ι
                                       С
                                        I
35
           N
             G
              R
                G
                  E
                   W
                                     Q
              KRHEEGHMLNC
                                T C
                                   F
                                       Q
           F
       N
        DT
             Н
                            QIGDSWEKY
                                          V H
       V D
          Q C
             QDSE
                   TGTF
                          Y
             RGIGEWHCQPLQTY
                                    PSSSGP
                                             V
       Y C
          ΥG
    I T E T P S Q P N S H P I Q W N A P Q P S H I S K Y I L
40
    RPVSIPPRNLGY
```

[SEQ ID NO: 1]

or a natural variant thereof from fibronectin in the manufacture of a reagent for diagnosing cancer.

46. Use of a reagent as defined in Claim 45, as a diagnostic agent.

- 47. A method of modulating cell migration the method comprising administering an effective amount of a polypeptide according to any one of Claims 10 to 13 to the site where modulation of cell migration is required.
 - 48. A method according to Claim 47, wherein the cell is a fibroblast or an endothelial cell.
 - 49. A method according to Claim 47 or 48, wherein the site is in a mammalian body.

10

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- 50. A method according to Claim 49, wherein the site is in a human body.
 - 51. Use of a polypeptide according to any one of Claims 10 to 13, in the manufacture of an agent for modulating cell migration.
- 20 52. Use of a polypeptide according to any one of Claims 10 to 13, for modulating cell migration.
 - 53. A method of healing a wound the method comprising administering to the locality of the wound an effective amount of a polypeptide according to any one of Claims 10 to 13.
 - 54. Use of a polypeptide according to any one of Claims 10 to 13, in the manufacture of a medicament for healing wounds.

55. Use of a polypeptide according to any one of Claims 10 to 13, for healing wounds.

- 56. A pharmaceutical composition comprising a polypeptide according to any one of Claims 10 to 13 and a pharmaceutically acceptable carrier.
 - 57. A polypeptide according to any one of Claims 10 to 13 for use in medicine.
- 10 58. A method of preventing scarring comprising administering to the locality of the site where scarring is to be prevented an effective amount of a polypeptide according to any one of Claims 10 to 13.

1/7 [SEQ ID NO: 2]

CAAACTTGGT GGCAACTTGC CTCCCGGTGC GGGCGTCTCT CCCCCACCGT CTCAA CATGC TTAGGGGTCC GGGGCCCGGG CTGCTGCTGC TGGCCGTCCA 101 GTGCCTGGGG ACAGCGGTGC CCTCCACGGG AGCCTCGAAG AGCAAGAGGC AGGCTCAGCA AATGGTTCAG CCCCAGTCCC CGGTGGCTGT CAGTCAAAGC AAGCCCGGTT GTTATGACAA TGGAAAACAC TATCAGATAA ATCAACAGTG GGAGCGGACC TACCTAGGCA ATGCGTTGGT TTGTACTTGT TATGGAGGAA GCCGAGGTTT TAACTGCGAG AGTAAACCTG AAGCTGAAGA GACTTGCTTT GACAAGTACA CTGGGAACAC TTACCGAGTG GGTGACACTT ATGAGCGTCC TAAAGACTCC ATGATCTGGG ACTGTACCTG CATCGGGGCT GGGCGAGGGA GAATAAGCTG TACCATCGCA AACCGCTGCC ATGAAGGGGG TCAGTCCTAC AAGATTGGTG ACACCTGGAG GAGACCACAT GAGACTGGTG GTTACATGTT AGAGTGTGTG TGTCTTGGTA ATGGAAAAGG AGAATGGACC TGCAAGCCCA 551 TAGCTGAGAA GTGTTTTGAT CATGCTGCTG GGACTTCCTA TGTGGTCGGA 601 GAAACGTGGG AGAAGCCCTA CCAAGGCTGG ATGATGGTAG ATTGTACTTG CCTGGGAGAA GGCAGCGGAC GCATCACTTG CACTTCTAGA AATAGATGCA ACGATCAGGA CACAAGGACA TCCTATAGAA TTGGAGACAC CTGGAGCAAG AAGGATAATC GAGGAAACCT GCTCCAGTGC ATCTGCACAG GCAACGGCCG AGGAGAGTGG AAGTGTGAGA GGCACACCTC TGTGCAGACC ACATCGAGCG GATCTGGCCC CTTCACCGAT GTTCGTGCAG CTGTTTACCA ACCGCAGCCT CTACTCTGTG GGGATGCAGT GGCTGAAGAC ACAAGGAAAT AAGCAAATGC TTTGCACGTG CCTGGGCAAC GGAGTCAGCT GCCAAGAGAC AGCTGTAACC

Fig. 1 (part 1)

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CAGACTTACG GTGGCAACTC AAATGGAGAG CCATGTGTCT TACCATTCAC 1101 1151 CTACAACGAC AGGACGGACA GCACAACTTC GAATTATGAG CAGGACCAGA AATACTCTTT CTGCACAGAC CACACTGTTT TGGTTCAGAC TCGAGGAGGA 1201 AATTCCAATG GTGCCTTGTG CCACTTCCCC TTCCTATACA ACAACCACAA 1251 TTACACTGAT TGCACTTCTG AGGGCAGAAG AGACAACATG AAGTGGTGTG 1301 GGACCACACA GAACTATGAT GCCGACCAGA AGTTTGGGTT CTGCCCCATG 1351 GCTGCCCACG AGGAAATCTG CACAACCAAT GAAGGGGTCA TGTACCGCAT 1401 TGGAGATCAG TGGGATAAGC AGCATGACAT GGGTCACATG ATGAGGTGCA 1451 CGTGTGTTGG GAATGGTCGT GGGGAATGGA CATGCATTGC CTACTCGCAG 1501 1551 CTTCGAGATC AGTGCATTGT TGATGACATC ACTTACAATG TGAACGACAC ATTCCACAAG CGTCATGAAG AGGGGCACAT GCTGAACTGT ACATGCTTCG 1601 1651 GTCAGGGTCG GGGCAGGTGG AAGTGTGATC CCGTCGACCA ATGCCAGGAT 1701 TCAGAGACTG GGACGTTTTA TCAAATTGGA GATTCATGGG AGAAGTATGT 1751 GCATGGTGTC AGATACCAGT GCTACTGCTA TGGCCGTGGC ATTGGGGAGT GGCATTGCCA ACCTTTACAG ACCTATCCAA GCTCAAGTGG TCCTGTCGAA 1801 GTATTTATCA CTGAGACTCC GAGTCAGCCC AACTCCCACC CCATCCAGTG 1851 GAATGCACCA CAGCCATCTC ACATTTCCAA GTACATTCTC AGGTGGAGAC 1901 CTGTGAGTAT CCCACCCAGA AACCTTGGAT ACTGAGTCTC CTAATCTTAT 1951 CAATTCTGAT GGTTTCTTTT TTTCCCAGCT TTTGAGCCAA CAACTCTGAT 2001 TAACTATTCC TATAGCATTT ACTATATTTG TTTAGTGAAC AAACAATATG 2051 TGGTCAATTA AATTGACTTG TAGACTGAAA AAAAAAAAA AAAAAAA 2101

Fig. 1 (part 2)

CHRCTITHTE SHEET (RIH F 26)

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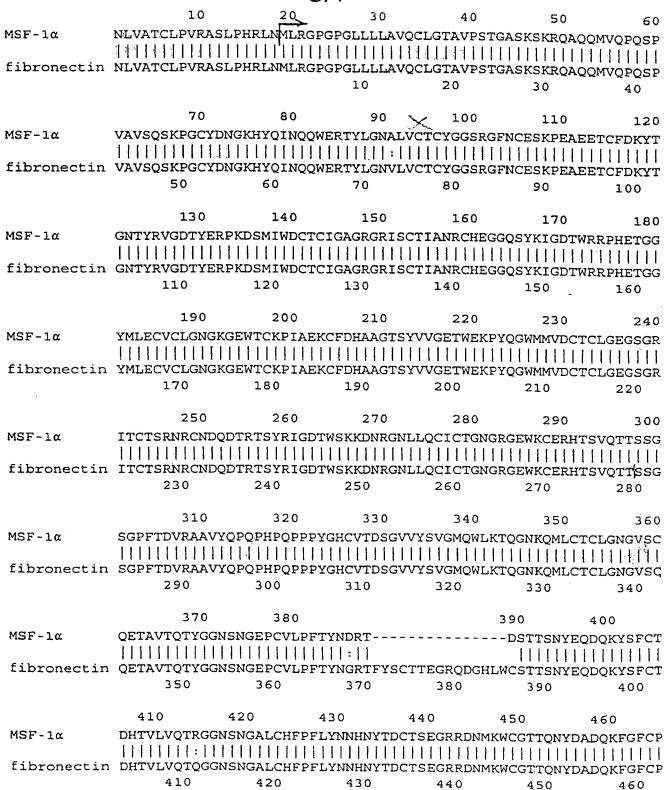


Fig. 2 (part 1)

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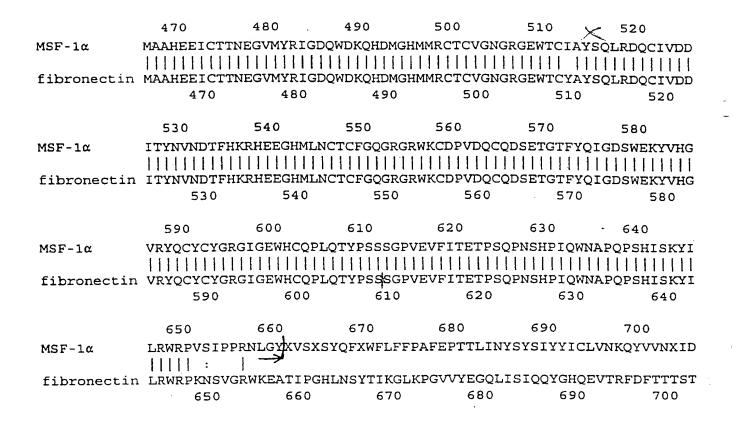


Fig. 2 (part 2)

	Sequence type:	Binding alte:
NLVATCLPVRASLPHRLN	5'untranslated region	
HLRGPGPGLLLLAVQCLGTAVPSTGASKSKR	Signal	
¹³ О Л Q Q М И Q Р Q S Р И В И S Q S К Р G	NH,-terminal segment	
"Sey Sey " Cenx H Right nood Rife Ret y" L Shy a L V" Shit Shiy Shics R Shi F N gress K P B a B B T "Reference of Central Rife K D S " H I W D Great Ship Ship Ship R R I S gilt ia n R "Seyn B C " Gro Ship I ship Great R P H B T Bricy M L B Chy Ship K B B W T gray P I A B K "I F Shi A A Shit Ship V Phe Rhaip K N P Y O Shaw H W D Ship Great I fift s R N R "I Reference C Ship Ship Ship K D N R Gray M L L O Griff R R P R R Gray B C L D Griff R R R R R R R R R R R R R R R R R R	H H H H H	Fibrin Heparin S.aureus
""H T S V Q T T S S G S G P F T D V R A A V Y Q P Q P H P Q P P P Y G H	Connecting strand	
""GBN T D S ^ 유명 V MHS V 유명사 Q MHL KSH * * * * * * * * * * * * * * * * # # # # # # # # # # # # # # # # # # # #		5
"y a v t beten gegeneralises p giv l beret kindd r t - · · · · · · · · · · · · · b s bepys blode q bloding s blody d h "y, v l v beter gegeneralises p giv l beret kind n n n t d c tes b g ren den n h k m charen night a beret b a		Gelätin Gelät
op ттие ° g-yv м k/k т g-pp o H p k o н p ° м g iн м м к ggt g iv g iv g iv g iv g iv g in v s o ь к b o мo т v b ° ° b т т kin v v p j т к и к к к н е ° в g-jn м ь и g iv g iv g iv g iv g ip v b o мeg o b s в т g ir в ij o <u>т g is iv s ki</u> ne kin ° ° v v н g iv s iv v g iv g iv g iv g iv g iv g	ммм	
***S G P V E V F I T E TIPS O P N SH P ** THO BIN A BNO P S H TIS K KAI L R H R B	111	
"IVS IPPRNEGY"	Unique sequence	
· V S · S Y Q F · W F L F F P A F E P T T L I N Y S Y S I Y Y I C L V N K Q Y V V N • I D L • T E K K K K K K	3'untranslated region	

Fig.

6/7

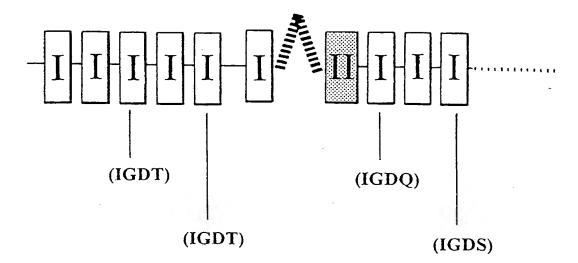
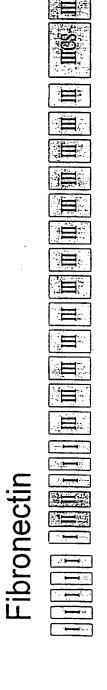


Fig. 4

7/7



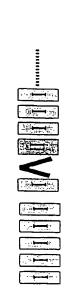


Fig. 5





(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	/Form PCT/ISA/220) as well as where applicable, item 5 below				
DUNW/P20111PC	ACTION	· · · · · · · · · · · · · · · · · · ·			
International application No.	International filing date (day/month/year)	(Earliest) Priority Date (day/month/year)			
PCT/GB 98/ 03766 15/12/1998 16/12/1997					
Applicant					
	_				
UNIVERSITY OF DUNDEE et a	Ι.				
This International Search Report has been according to Article 18. A copy is being tra	n prepared by this International Searching Auth Ansmitted to the International Bureau.	nority and is transmitted to the applicant			
This International Search Report consists It is also accompanied by	of a total of sheets. a copy of each prior art document cited in this	report.			
Basis of the report					
	international search was carried out on the bases otherwise indicated under this item.	sis of the international application in the			
Authority (Rule 23.1(b)).	as carried out on the basis of a translation of the				
b. With regard to any nucleotide an was carried out on the basis of the		ternational application, the international search			
	nal application in written form.				
}	rnational application in computer readable for	n.			
· · · ·	this Authority in written form.				
	this Authority in computer readble form.	and the first party and the discharge in the			
	sequently furnished written sequence listing d s filed has been furnished.	oes not go beyond the disclosure in the			
the statement that the info furnished	ormation recorded in computer readable form is	s identical to the written sequence listing has been			
2. X Certain claims were four	nd unsearchable (See Box I).				
3. Unity of invention is lace	king (see Box II).				
4. With regard to the title,					
X the text is approved as su	bmitted by the applicant.				
the text has been establis	hed by this Authority to read as follows:				
E. With regard to the chetrost					
5. With regard to the abstract, the text is approved as su	bmitted by the applicant				
the text has been establis	hed, according to Rule 38.2(b), by this Authorie date of mailing of this international search rep	ty as it appears in Box III. The applicant may, port, submit comments to this Authority.			
6. The figure of the drawings to be publ	1				
X as suggested by the appli	cant.	None of the figures.			
because the applicant fail	· · · · · · · · · · · · · · · · · · ·	•			
because this figure better	characterizes the invention.				



International application No.

PCT/GB 98/03766

Box Observations where certain claims were found unsearchable (Continuation of item 1 of itest sheet)
This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:
1. X Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely: see FURTHER INFORMATION sheet PCT/ISA/210
2. Claims Nos.: because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).
Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)
This International Searching Authority found multiple inventions in this international application, as follows:
see additional sheet PCT/ISA/210
As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. X As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
Remark on Protest The additional search fees were accompanied by the applicant's protest. No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-18, 25, 27, 29, 31, 33 and 36-58 in totality, and 24, 34 and 35 partly

Polynucleotide and polypeptide of migration stimulating factor and their uses, and an antibody reactive with the polypeptide, but not with fibronectin, and the use of the antibody.

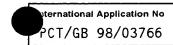
2. Claims: 19-23, 26, 28, 30 and 32 in totality, and 24, 34 and 35 partly

An antibody reactive with fibronectin but not with the polypeptide of invention $I,\ \mbox{and}\ \mbox{its}$ use.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Although claims 47-50, 52, 53, 55 and 58 are directed to methods of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy



A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/12 C12N5/10 C12
C12Q1/68 G01N33/574 A61

C12N15/63 A61K38/39 C07K14/78 C07K16/18

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 CO7K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

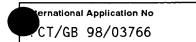
C. DOCUM	ENTS CONSIDERED TO BE RELEVANT	
Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Х	WO 94 16085 A (ZYMOGENETICS INC) 21 July 1994	1-3, 6-13,27, 29,53-57
Α	see abstract; claims	4,5,51, 52
	see page 2, line 30 - page 4, line 8	
Α	WO 90 00567 A (CANCER RES CAMPAIGN TECH) 25 January 1990	9-18,25, 27,29, 36-39, 44-57
	see page 1 - page 10	'' ''
	-/	
		•

Further documents are listed in the continuation of box C.	χ Patent family members are listed in annex.		
"A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. "8" document member of the same patent family		
Date of the actual completion of the international search	Date of mailing of the international search report		
20 May 1999	07/06/1999		
Name and mailing address of the ISA	Authorized officer		
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Ceder, O		

	uation) DOCUMENTS CONSIDERED TO BE RELEVANT	
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KORNBLIHTT ET AL.: "Human mRNA for fibronectin" EMBL SEQUENCE DATABASE,7 November 1985, 'XP002103220 HEIDELBERG DE Ac X02761	1-3,6
X	see the whole document -& KORNBLIHTT ET AL.: "Primary structure of human fibronectin: differential splicing may generate at least 10 polypeptides from a single gene" THE EMBO JOURNAL, vol. 4, no. 7, 1985, pages 1755-1759, VXP002051533 see abstract see page 1759, left-hand column	1-3,6, 10-13, 27,29
X	KORNBLIHTT ET AL.: "Human fibronectin precursor" SWISSPROT SEQUENCE DATA BASE,21 July 1986, XP002103221 Ac P02751	10-13, 27,29
X	see the whole document & KORNBLIHTT ET AL.: "Primary structure of human fibronectin: differential splicing may generate at least 10 polypeptides from a single gene" THE EMBO JOURNAL, vol. 4, no. 7, 1985, pages 1755-1759, VXP002051533 see abstract see page 1759, left-hand column	1-3,6, 10-13, 27,29
X	EP 0 207 751 A (DELTA BIOTECHNOLOGY LTD) 7 January 1987 see abstract; claims; figures 2,3 see page 13, line 30 - page 15, line 10	1,3, 6-10,12, 13,27,29
X	"Homo sapiens fibronectin splice form ED-A" PIR1 SEQUENCE DATA BASE,27 November 1985, XP002103253 Ac FNHU see the whole document & DEAN ET AL.: "Cloning and analysis of the promoter region of the human fibronectin gene" PROC. NATL. ACAD. SCI. U.S.A., vol. 84, 1987, pages 1876-1880,	10-13, 27,29
X	/ EP 0 344 134 A (IST NAZ RIC SUL CANCRO) 29 November 1989 see abstract; figure 1 -/	19-24, 26,28,30

1





	ation) DOCUMENTS CONSIDERED TO BE RELEVANT	
Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5 571 679 A (SEKIGUCHI KIYOTOSHI ET AL) 5 November 1996	14-17, 24,25,27
A	US 5 629 291 A (RUOSLAHTI ERKKI I ET AL) 13 May 1997 see abstract see column 1, line 29 - line 40 see column 1, line 55 - line 57	10,29, 36,47, 48,53-55
	•	
·		

1

mation on patent family members

ternational Application No PCT/GB 98/03766

	atent document d in search report		Publication date	<u> </u>	Patent family member(s)	Publication date
WO	9416085	Α	21-07-1994	US	5830700 A	03-11-1998
WO	9000567	Α	25-01 - 1990	EP	0423207 A	24-04-1991
				JP	3505732 T	12-12-1991
EP	0207751	Α	07-01-1987	AT	58381 T	15-11-1990
				AU	603059 B	08-11-1990
				ΑU	5931586 A	08-01-1987
				DK	306386 A	29-12-1986
				FI	862756 A	29-12-1986
				JP	62089699 A	24-04-1987
EP	0344134	Α	29-11-1989	AT	100471 T	15-02-1994
				DE	68912403 D	03-03-1994
				DE	68912403 T	11-05-1994
US	5571679		05-11-1996	 EP	0580859 A	02-02-1994
				WO	9217604 A	15-10-1992
US	5629291	Α	13-05-1997	US	5453489 A	26-09-1995
				US	5747452 A	05-05-1998
				US	5837813 A	17-11-1998
				AT	152173 T	15-05-1997
				AU	3656893 A	01-09-1993
				CA	2129115 A	05-08-1993
				DE	69310145 D	28-05-1997
				ÐK	624196 T	03-11-1997
				EP	0624196 A	17-11-1994
				FI	943568 A	29-07-1994
				JP	7506342 T	13-07-1995
				NO	942825 A	29-09-1994
				WO	9315203 A	05-08-1993

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

					<u> </u>	
Applicant's or agent's file reference			See Notification of Transmittal of International FOR FURTHER ACTION Preliminary Examination Report (Form PCT/IPEA/416)			
DUNW/P2	0111	PC				
International application No.				(month/year)	Priority date (day/month/year)	
	T/GB98/03766 15/12/1998 16/12/1997					
International	Patent	Classification (IPC) or na	tional classification and IPC			
C12N15/1	2					
Applicant						
	ITY C	OF DUNDEE et al.				
				anarod by this Inte	ernational Preliminary Examining Authority	
1. This in	ternat	ional preliminary exam	ination report has been pro according to Article 36.	epared by this into	ernational Preliminary Examining Authority	
and is	uansi	miled to the applicant				
		OT consists of a total o	f 7 sheets, including this co	over sheet.		
 ⊠ Th	nis rep	ort is also accompanie	ed by ANNEXES, i.e. sheet	s of the description	on, claims and/or drawings which have	
l ha		nanded and are the ha	isis for this report and/or sh 607 of the Administrative In	ieets containing i	ecuncations made belove and	
(s	ee Ru	lie 70.16 and Section (DOT OF THE AGITHIBATATIVE III		•	
These	anne	xes consist of a total of	f 16 sheets.		·	
	**					
į						
3. This r	eport	contains indications re	lating to the following items	••		
1 .	\boxtimes	Basis of the report				
11		Priority				
111		Non-establishment of	opinion with regard to nove	elty, inventive ste	p and industrial applicability	
IV		Lack of unity of inven	tion			
V	☒	Reasoned statement citations and explana	under Article 35(2) with reg tions suporting such staten	gard to novelty, in nent	ventive step or industrial applicability;	
VI		Certain documents of				
VII		Certain defects in the	international application	tion		
VIII	\boxtimes	Certain observations	on the international applica	аноп		
Date of su	bmissi	on of the demand		Date of completion		
16/07/1999			s on oo			
16/07/19	999	•			10.00	
Nama and	l mailin	g address of the internation	onal	Authorized officer	A PARTY SAN COLOR	
preliminary	y exam	nining authority:			(
31	Eur	opean Patent Office 0298 Munich		Sprinks, M		
<i>9</i>))	Tel	. +49 89 2399 - 0 Tx: 523	656 epmu d	•	Tax Marine Marine	
	Fax	c: +49 89 2399 - 4465		Telephone No. +49	89 2399 8706	

INTERNATIONAL PRELIMINARY **EXAMINATION REPORT**

International application No. PCT/GB98/03766

١.	Basis	of the	report
----	--------------	--------	--------

1. This report has been drawn on the basis of (substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.): Description, pages:

1-52 Claims, No.: 31/03/2000 with letter of 31/03/2000 as received on 1-52

Drawings, sheets:

1/7-7/7

as originally filed

as originally filed

2. The amendments have resulted in the cancellation of:

the description,

pages:

★ the claims,

Nos.:

53-58

☐ the drawings,

sheets:

This report has been established as if (some of) the amendments had not been made, since they have been 3. 🖾 considered to go beyond the disclosure as filed (Rule 70.2(c)):

see separate sheet

4. Additional observations, if necessary:

INTERNATIONAL PRELIMINARY **EXAMINATION REPORT**

International application No. PCT/GB98/03766

V. Reasoned statement under Article 35(2) with regard to nov lty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)

Yes:

Claims 1-18,21,23-25,27,31-52

No:

Yes:

Claims 19,20,22,26,28-30

Claims 1-18,21,23-25,27,31-52

No:

Claims

Industrial applicability (IA)

Inventive step (IS)

Yes:

Claims 1-52 Claims

No:

2. Citations and explanations

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

The following documents (D) are mentioned for the first time in this opinion/report; the numbering will be adhered to in the rest of the procedure:

D1...WO 94 16085 A (ZYMOGENETICS INC) 21 July 1994 D2...EMBO J., vol. 4, no. 7, 1985, pages 1755-1759 (Kornblihtt et al.) D3...EMBL Data library, database Pir2, accession no. S14428 (fibronectin precursor - rat), 1989 (Hynes et al.) (document cited from the examiner's own knowledge. A copy of the document is annexed to the communication). D4...EP-A-0 344 134 (IST NAZ RIC SUL CANCRO) 29 November 1989 D5...DATABASE ENTRY, ID: FINC_XENLA, SWISSPROT database, 1 November 1997 (Desimone et al.) (document cited from the examiner's own knowledge and for applicant's information only. A copy of the document is annexed to the communication).

D6...US-A-5 571 679 (SEKIGUCHI KIYOTOSHI ET AL) 5 November 1996

Basis of the opinion/report

Unallowable amendments

In amended claims 14-17, 21-23, 26, 27 and 30-37, filed on 31.03.00, the 1) applicant has attempted to differentiate antibodies and probes etc. for detecting MSF of the present invention from those for detecting fibronectin of the prior art by using phrasing such as "not reactive towards a fibronectin which contains the amino acid sequence FY...WC". However, it should be noted that this expression effectively narrows the disclaimer (i.e.broadens the subject-matter claimed) in a way which is unclear and lacks basis in the application as originally filed. The text upon which the applicant has based these amendments only actually concerns the generation of antibodies against fibronectin, and refers to an oligopeptide sequence of which FY...WC is only a part (see page 24 of the present description).

In any event, as mentioned above, claims containing such a disclaimer would still be considered unclear since the antibodies and probes claimed could still be reactive against a fibronectin with a single amino acid substitution at one of the positions indicated (see for example the fibronectin of D5).

EXAMINATION REPORT - SEPARATE SHEET

Consequently, said claims have been interpreted as if the disclaimer only referred to fibronectin in general.

V) Reasoned statement

Novelty

- The present application does not satisfy the criterion set forth in Article 33 (2) PCT because the subject-matter of claims 19, 20, 22, 26 and 28-30 is not new in respect of prior art as defined in the regulations (Rule 64.1 - 64.3 PCT).
- 2) Said claims refer to antibodies and probes which are characterised only by their ability to react or hybridise with fibronectin polypeptides and polynucleotides of the prior art but not with the MSF polypeptides and polynucleotides of the present invention. Consequently, in view of the fact that antibodies and probes reactive or hybridising with fibronectin and its other splice variants are disclosed throughout the prior art (e.g. D1-D6) and that MSF polypeptide and polynucleotide sequences were not available before the priority date of the present application, this definition alone must, for the time being, be considered insufficient to establish the novelty of the claimed antibodies and probes over those of said documents.
 - If, however, at a later date, comparative studies were to demonstrate that the antibodies and probes claimed are indeed novel, they might also be considered unitary with the other subject-matter claimed, since a broad unifying and inventive concept such as "differentiating MSF from fibronectin" might be acknowledged (method claims referring to the use of MSF polypeptides and polynucleotides for screening fibronectin/non-MSF-reactive antibodies and probes might be another alternative).

Novelty, inventive step and industrial applicability

Claims 1-18, 21, 23-25, 27 and 31-52 are considered to fulfil the criteria of Article 33 (2) - (4) PCT since, in the light of the available prior art, they define what appears to be new, inventive and industrially applicable concept, namely a splice variant of fibronectin (MSF), defined by its sequence in SEQ ID NO:1 and

EXAMINATION REPORT - SEPARATE SHEET

encoded by the nucleotide sequence of SEQ ID NO:2 (as well as variants thereof differing only in the usage of the genetic code).

Nevertheless, particular attention should be paid to the objections concerning lack of clarity made in section VIII below.

Industrial applicability

For the assessment of the subject-matter of present claims 41-44, 46, 47, 49 and 4) 52 (as far as in vivo methods are concerned) on the question whether it is industrially applicable, no unified criteria exist in the PCT. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but will allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

VIII) Certain observations

Insufficiency of disclosure and clarity

- The present application does not satisfy the criterion set forth in Articles 5 and 6 1) PCT because the subject-matter of claims 1-4, 11, 14-19, 21-23, 26, 27, 30-32 and 34-37 is insufficiently disclosed and/or unclear.
- As mentioned in section V above, the invention appears to centre around one 2) particular fibronectin splice variant, MSF. Consequently, use of the open-ended term "encoding" in claim 1 causes considerable problems with regard to the clarity and sufficiency of disclosure of the subject-matter of claims 1-4 for the following reasons:

D2 discloses that fibronectin and other splice variants thereof with MSF activity were known in the prior art and that all are encoded by a common fibronectin gene - a point confirmed on page 4 of the present application. As a result, claims broadly directed to a(ny) polynucleotide encoding one fibronectin variant also

encompass the full length fibronectin gene and unspliced mRNA which also encodes all the others! Whether the polynucleotide is said to be "recombinant" or not is irrelevant - the present claims cover subject-matter which goes far beyond the inventive concept of the present invention and is not unitary with it. In order to fulfil the requirements of the PCT with regard to clarity and sufficiency of disclosure, the scope of protection sought should be commensurate with the

In this case, the applicant has only isolated <u>one specific fibronectin variant</u> and one polynucleotide sequence which encodes it - the claims should reflect this.

technical contribution which the application as a whole makes to the art.

- Claims 2, 3, 11 and 30-32 still refer to unspecified <u>"variants"</u>, <u>fragments"</u>, <u>"derivatives" and "fusions"</u> which need not retain any of the properties which characterise the polynucleotides or polypeptides to which they ultimately refer.
- 4) "Fibronectin" is itself a vague term (D2 highlights this point describing fibronectins as <u>a class</u> of proteins), such that even MSF of the present invention could be considered to be a "fibronectin", bringing the clarity, novelty and overall relevance of claims simply referring to "fibronectin" into question.
- In order to be clearly defined and belong to the inventive concept of the present invention, all the antibodies referred to in **claims 14-19 and 21-23** would need to be <u>specific</u> for the polypeptide against which they were raised. Otherwise, said claims may be considered to cover cross-reacting antibodies of the prior art.
 - Similarly, **claims 26 and 27** are unclear because the "stringent hybridization conditions" mentioned in the claims are not precisely defined therein. It is therefore left entirely up to third parties to decide which particular conditions to use.
- 6) Claims 30-32 and 34-37 are unclear because they refer to methods for detecting MSF using antibodies and probes which detect <u>fibronectin</u> (see claim dependencies).



CLAIMS

30

1. A recombinant polynucleotide encoding a polypeptide consisting of (1) the amino acid sequence

```
NLVATCLPVRASLPHRLN
5
   MLRGPGPGLLLLAVQCLGTAVPSTGASKSK
   RQAQQMVQPQSPVAVSQSKPGCYDNGKHYQ
   INQQWERTYLGNALVCTCYGGSRGFNC
   P E A E E T C F D K Y T G N T Y R V G D T Y E R P K D
   WDCTCIGAGRGRISCTIANRCHEGGQS
10
    D T W R R P H E T G G Y M L E C V C L G N G K G E W T
    1 A E K C F D H A A G T S Y V V G E T W E K P Y Q G
   V D C T C L G E G S G R I T C T S R N R C N D Q D T R
   RIGDTWSKKDNRGNLLQCICTGNGRGEWKC
   ERHTSVQTTSSGSGPFTDVRAAVYQPQ
15
   Q P P P Y G H C V T D S G V V Y S V G M Q W L K T Q G
   MLCTCLGNGVSCQETAVTQTYGGNSNG
   V L P F T Y N D R T D S T T S N Y E Q D Q K Y S F C T
   V L V Q T R G G N S N G A L C H F P F L Y N N H N Y T
                        QNYDADQKFGFC
   SEGRRDNMKWCGTT
20
   HEEICTTNEGVMYRIGDQWDKQHDMGHMMR
    T C V G N G R G E W T C I A Y S Q L R D Q C I V D D I T Y
   NVNDTFHKRHEEGHMLNCTCFGQGRGRWKC
   D P V D Q C Q D S E T G T F Y Q I G D S W E K Y V H G
   Q C Y C Y G R G I G E W H C Q P L Q T Y P S S S G P V E V F
25
    TETPSQPNSHPIQWNAPQPSHISKYILRW
   RPVSIPPRNLGY
```

or consisting of (2) a variant or fragment or derivative of said amino acid sequence which has migration stimulating factor activity and which includes the amino acid sequence VSIPPRNLGY, PCVLPFTYNDRTD, DRTDSTTSNYEQDQ, TDHTVLVQTR and/or REGNSNGALCH.

- 2. A polynucleotide according to Claim 1, wherein the polypeptide consists of the amino acid sequence shown in Figure 2 labelled pMSF1α between positions 19 and 660, or a variant or fragment or derivative of said amino acid sequence.
- 3. A recombinant polynucleotide encoding a fusion of a polypeptide as defined in Claim 1 or 2 and another polypeptide.

- 4. A polynucleotide according to Claim 1 or 2, which contains no introns.
- 5 5. A polynucleotide according to any one of the preceding claims, comprising the polynucleotide whose sequence is shown in Figure 1.
 - 6. A polynucleotide according to any one of the preceding claims, comprising the polynucleotide whose sequence is shown in Figure 1 between positions 57 and 1982.

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- 7. A replicable vector comprising a polynucleotide as defined in any one of Claims 1 to 6.
- 8. A host cell comprising a recombinant polynucleotide or a replicable vector as defined in any one of Claims 1 to 7.
- 9. A method of making a polypeptide as defined in Claim 1 or 2 or a fusion of said polypeptide and another polypeptide the method comprising culturing a host cell as defined in Claim 8 which expresses said polypeptide or fusion and isolating said polypeptide or fusion from said host cell culture.
 - 10. A polypeptide consisting of (1) the amino acid sequence

ERHTSVQTTSSGSGPFTDVRAAVYQPQPHP D S G V V Y S V G M Q W L K T Q G N YGHC V T P P Ρ SCQETAVTQTYGGNSNGE GNGV С Т C L P F T Y N D R T D S T T S N Y E Q D Q K Y S F C T D H T CHFPFLYNNHNYTDCT A L V L V Q T R G G N S N G 5 Q N Y D A D Q K F G F C P M A AС Т Т SEGRRDNMKW G YRIGDQWDKQHDMGHMMR V М HEEICTTNEG IAYSQLRDQCIVDDITY CTCVGNGRGE WT С HMLNCTCFGQGRGRWKC NVNDTFHKRHE Ε G QIGDSWEKYVHG 10 CYCYGRGIGEWHCQPLQTYPSSSGPVEVF TETPSQPNSHPIQWNAPQPSHISKYILRW RPVSIPPRNLGY

- or consisting of (2) a variant or fragment or derivative of said amino acid sequence which has migration stimulating factor activity and which includes the amino acid sequence VSIPPRNLGY, PCVLPFTYNDRTD, DRTDSTTSNYEQDQ, TDHTVLVQTR and/or REGNSNGALCH.
- 20 11. A polypeptide according to Claim 10, consisting of the amino acid sequence shown in Figure 2 labelled pMSF1α between positions 19 and 660, or a variant or fragment or derivative of said amino acid sequence.
- 12. A fusion of a polypeptide according to Claim 10 or 11 and another polypeptide.
 - 13. A polypeptide obtainable by the method of Claim 9.
- 30 14. An antibody reactive towards the polypeptide whose amino acid sequence is

N L V A T C L P V R A S L P H R L N

M L R G P G P G L L L L A V Q C L G T A V P S T G A S K S K R Q A Q Q M V Q P Q S P V A V S Q S K P G C Y D N G K H Y Q S I N Q Q W E R T Y L G N A L V C T C Y G G S R G F N C E S K P E A E E T C F D K Y T G N T Y R V G D T Y E R P K D S M I W D C T C I G A G R G R I S C T I A N R C H E G G Q S Y K I G D T W R R P H E T G G Y M L E C V C L G N G K G E W T C K

PIAEKCFDHAAGTSYVVGETWEKPYQGWMM T C L G E G S G R I T C T S R N R C N D Q D T R T TWSKKDNRGNLLQCICTGNGRGEWKC Q T T S S G S G P F T D V R A A V Y Q P Q P H P V S H C V T D S G V V Y S V G M Q W L K T Q G N K Q P P ΥG G N G V S C Q E T A V T Q T Y G G N S N G E L YNDRTDSTTSNYEQDQKYSFCTDHT GGNSNGALCHFPFLYNNHNYTDCT T R RRDNMKWCGTTQNYDADQKFGFCPMAA G TNEGVMYRIGDQWDKQHDMGHMMR 10 Ι T GNGRGEWTCIAYSQLRDQCIVDDITY T C v NDTFHKRHEEGHMLNCTCFGQGRGKWKC YQIGDSWEKYVHGVRY PVDQCQDSETG T F YGRGIGEWHCQPLQTYPSSSGPVEVF Ī T E T P S Q P N S H P I Q W N A P Q P S H I S K Y I L R W 15 RPVSIPPRNLGY

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but not reactive towards a fibronectin which contains the amino acid sequence FYSCTTEGRQDGHLWC.

15. An antibody reactive towards the polypeptide whose amino acid sequence is shown in Figure 2 labelled pMSF1 α between positions 19 and 660 but not reactive towards a fibronectin which contains the amino acid sequence FYSCTTEGRQDGHLWC.

16. An antibody reactive towards an epitope present in the polypeptide whose amino acid sequence is

```
NLVATCLPVRASLPHRLN
   M L R G P G P G L L L L A V Q C L G T A V P S T G A S K S K
   RQAQQMVQPQSPVAVSQSKPGCYDNGKHYQ
30
                           CYGGSRGFNC
   INQQWERTYLGNALVC
                         T
                         RVGDTYERPKD
    EAEETCFDKYTGNTY
                           ANRCHEGGQ
   WDCTCIGAGRGRISC
                        Т
                          Ι
                            CLGNGKGEWT
                           V
   GDTWRRPHETGGYMLEC
    IAEKCFDHAAGTSYVVGETWEKP
35
   V D C T C L G E G S G R I T C T S R N R C N D Q
    IGDTWSKKDNRGNLLQC
                            ICTGNG
                                     RGEWKC
                         T D V R A A V Y
   ERHTSVQTTSSGSGPF
   Q P P P Y G H C V T D S G V V Y S V G M Q W L K
   MLCTCLGNGVSCQETAVTQTYGGN
40
      P F T Y N D R T D S T T S N Y E Q D Q K Y S
                                     F
   V L V Q T R G G N S N G A L C H F P F L Y N N H N Y
   SEGRRDNMKWCGTTQNYDADQKFGFC
   H E E I C T T N E G V M Y R I G D Q W D K Q H D M G H M M R
   CTCVGNGRGEWTCIAYSQLRDQCIVDDITY
45
```

N V N D T F H K R H E E G H M L N C T C F G Q G R G R W K C
D P V D Q C Q D S E T G T F Y Q I G D S W E K Y V H G V R Y
Q C Y C Y G R G I G E W H C Q P L Q T Y P S S S G P V E V F
I T E T P S Q P N S H P I Q W N A P Q P S H I S K Y I L R W
R P V S I P P R N L G Y

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but which epitope is not present in a fibronectin which contains the amino acid sequence FYSCTTEGRQDGHLWC.

- 17. An antibody reactive towards an epitope present in the polypeptide whose amino acid sequence is shown in Figure 2 labelled pMSF1α between positions 19 and 660 but which epitope is not present in a fibronectin which contains the amino acid sequence FYSCTTEGRQDGHLWC.
- 18. An antibody according to any one of Claims 14 to 17, reactive towards a molecule comprising any one of the peptides ISKYILRWRPVSIPPRNLGY or EPCVLPFTYNDRTDSTTSNYEQDQ or CTDHTVLVQTRGGNS-NGALCH.
 - the peptides of towards any one reactive antibody 19. An **EPCVLPFTYNGRTFYSCTTEG-QQWERTYLGNVLVCTCYGGSR** or CTDHTVLVQTQGGNSNGALCH RQDGHLWCSTTSNYEQDQ or VGNGRGEWTCYAYSQLRDQCI or ISKYILRWRPKNSVGRWKEA.
 - 20. An antibody according to any one of Claims 14 to 19 which is a monoclonal antibody.
- 21. A method of making an antibody which is reactive towards the polypeptide whose amino acid sequence is

NLVATCLPVRASLPHRLN C L G T A V P S T G A S K S K MLRGPGPGLLLLAVQ Q S K P G C Y D N G K H Y Q QAQQMVQPQSPVAV S CYGGSRGFNCESK NQQWERTYLGNALVC T VGDTYERPKDSMI Y EAEETCFDKYTG R N Т ANRCHEGGQ DCTCIGAGRGRI s c Т Ι LGNGKGEWTCK E С V С DTWRRPHETGG Y M L PYQG IAEKCFDHAAG Т S Y V V GE Т WEK QDT V D C T C L G E G S G R I T C TSRN R С N D RIGDTWSKKDNRGNL G R L Q С Ι С Т G N 10 Ρ RHTSVQTTSSGSGFF ΑV ΥQ Т D V R A PPYGHCVTDSGVVY T Q SVGMQWL K MLCTCLGNGVSCQETAVTQ S TYGG N N LPFTYNDRTDSTTSNY EQDQKYS F С LVQTRGGNSNGALCHFPFLYNNH Y 15 EGRRDNMKWCGTTQNYDADQKF G F ICTTNEGVMYRIGDQWDKQH D VGNGRGEWTCIAYSQLRDQCI V D DTFHKRHEEGHMLNCTCFGQG R G V Q C Q D S E T G T F Y Q I G D S W E K Y V H 20 V D P YGRGIGEWHCQPLQTYPSSSGPVE С Y C PSQPNSHPIQWNAPQPSHISKYILRW Т PVSIPPRNLGY

25 (MSF) and which is not reactive with a fibronectin which contains the amino acid sequence FYSCTTEGRQDGHLWC, the method comprising the steps of, where appropriate, immunising an animal with a peptide which distinguishes MSF from a fibronectin which contains the amino acid sequence FYSCTTEGRQDGHLWC and selecting an antibody which binds MSF but does not substantially bind a fibronectin which contains the amino acid sequence FYSCTTEGRQDGHLWC.

22. A method of making an antibody which is reactive towards fibronectin which contains the amino acid sequence FYSCTTEGRQDGHLWC and which is not reactive towards the polypeptide whose amino acid sequence is

NLVATCLPVRASLPHRLN M L R G P G P G L L L A V Q C L G T A V P S T G A S K S K Q M V Q P Q S P V A V S Q S K P G C Y D N G K H Y YLGNALVCTCYGGSRGFNC R T QQWE D K Y T G N T Y R V G D T Y E R P K D T C F 40 E Ε I G A G R G R I S C T I A N R C H E G G Q С T RRPHETGGYMLECVCLGNGKGEWT T W EKCFDHAAGTSYVVGETWEKPYQGWMM D C T C L G E G S G R I T C T S R N R C N D Q D T R T S Y

RIGDTWSKKDNRGNLLQCICTGNGRGE RHTSVQTTSSGSGFF V ΥQ Т D VRAA C V T D S G V VY S VGMQ W L K Q QETAVT Q T Y G G N S N GNGVSC YNDRTDSTTSNY D K Y S F E Q Q 5 TRGGNSNGALCHFPFLYN N NMKWCGTTQNYDADQK F F G R R D TNEGVMYRIGDQWDKQH D С \mathbf{T} GNGRGEWTCIAYSQLRDQC I V D V DTFHKRHEEGHMLNCTCFGQG R G 10 QCQDSETGTFYQIGDSWEKYV D YGRGIGEWHCQPLQTYPSSSGPVE Q P N S H P I Q W N A P Q P S H I S K Y I L R W PS RPVSIPPRNLGY

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(MSF), the method comprising the steps of, where appropriate, immunising an animal with a peptide which distinguishes a fibronectin which contains the amino acid sequence FYSCTTEGRQDGHLWC from MSF and selecting an antibody which binds a fibronectin which contains the amino acid sequence FYSCTTEGRQDGHLWC but does not substantially bind MSF.

23. A molecule which is capable of, following immunisation of an animal if appropriate, giving rise to antibodies which are reactive towards the polypeptide whose sequence is

```
NLVATCLPVRASLPHRLN
25
                   PGLLLLAVQCLGTAVPSTGASKSK
     MLRGPG
                    \begin{smallmatrix} V \end{smallmatrix} Q \end{smallmatrix} P \begin{smallmatrix} Q \end{smallmatrix} S \end{smallmatrix} P \begin{smallmatrix} V \end{smallmatrix} A \begin{smallmatrix} V \end{smallmatrix} S \end{smallmatrix} Q \_S \end{smallmatrix} K \end{smallmatrix} P \end{smallmatrix} G \end{smallmatrix} C \begin{smallmatrix} Y \end{smallmatrix} D \end{smallmatrix} N \end{smallmatrix} G \end{smallmatrix} K \end{smallmatrix} H \end{split} Y
              O M
                       YLGNALVCTCYGGSRGFNC
              WERT
                                 GNTYRVGDTYERPKD
                        D K Y T
                T
                   С
                     F
              Ε
                          RGRISCTIANRCHEGGGQS
                        G
30
              С
                 Ι
                   G
                     Α
                                 YMLECVCLGNGKGEWT
                            G G
                       E
                          \mathbf{T}
                   P
                     Η
              R
                                 T S Y V V G E T W E K P Y Q G
                            A G
                          Α
                 С
                   F
                     D
                       Η
              Κ
                                   T C T S R N R C N D Q D T R T
                        G
                          S G
                              R I
                   G
                     Ε
              С
                 L
            T
                            NRGNLLQCICTGNGRGEW
                        Κ
                          D
                 W
                   S
                     K
          G
            D
                                   G P F T D V R A A V Y Q P
                              G S
35
              S
                 ν
                   Q
                     Т
                        Т
                          S
                            S
         Н
            T
                            D S G V V Y S V G M Q W L K T Q G
                          Т
         Ρ
            P
              Y
                 G
                   Η
                     С
                        V
                            SCQETAVTQTYGGNSNG
                     NG
                          V
            Т
               C
                 L
                   G
                                   T S N Y E Q D Q K Y S F
                            D S T
                          T
         Р
            F
              Τ
                 YN
                     D R
                                             PFLYNNHNYT
                               G A
                                    L C H F
                          S
                 R
                   G
                      G N
                            N
         V
            Q
              Т
                                    TQNYDADQKFGFC
                             CG
                                 Т
                 D
                     МK
                          W
40
            R R
                   Ν
         G
                                        G D Q W D K Q H D M G H M M R
                                    RΙ
                      ΝE
                          G
                            V
                               M Y
            Ι
              С
                 T
                   T
                                        Y S Q L R D Q C I V D D I T
                                 С
                          E W
                               Т
                                    Ι
                                      Α
            V G
                 ΝG
                      R G
                                        LNCTCFGQGRGRWKC
                               E
                                 G
                                    н м
                 FHKR
                          Н
                             Ε
         и D Т
                                        QIGDSWEKYVHGVRY
                                    F
                               G T
                                      Y
                      D
                        S
                          Ε
                             Т
                 С
                   Q
          V
            D
                                    CQPLQTYPSSSGPVEVF
          YCYGRGI
                          GEWH
45
      ITETPSQPNSHPIQWNAPQPSHISKYILRW
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but not reactive towards a fibronectin which contains the amino acid sequence FYSCTTEGRQDGHLWC.

- 24. A peptide consisting of any one of the sequences

 QQWERTYLGNVLVCTCYGGSR or

 EPCVLPFTYNGRTFYSCTTEGRQDGHLWCSTTSNYEQDQ or

 CTDHTVLVQTQGGNSNGALCH or

 VGNGRGEWTCYAYSQLRDQCI or

 ISKYILRWRPKNSVGRWKEA.
 - 25. A molecule according to Claim 23 which is a peptide comprising any one of the sequences
- ISKYILRWRPVSIPPRNLGY; or
 PCVLPFTYNDRTDSTTSNYEQDQ; or
 TDHTVLVQTRGGNSNGALCH; or
 which are found in MSF.
- 26. A polynucleotide which is capable of hybridising at high stringency to a polynucleotide which encodes a fibronectin which contains the amino acid sequence FYSCTTEGRQDGHLWC but not a polynucleotide which encodes the polypeptide whose sequence is

Q P P P Y G H C V T D S G V V Y S V G M Q W L K T Q G N K Q YGGNSNG MLCTCLGNGVSCQETAVTQT KYSF VLPFTYNDRTDSTTS NYE Q D Q V L V Q T R G G N S N G A L C H F P F LYNNHN EGRRDNMKWCGTTQNYDAD KFGF Q ICTTNEGVMYRIGDQWDKQH D M VGNGRGEWTCIAYSQLRD Q C ΙV V N D T F H K R H E E G H M L N C T C F G Q V D Q C Q D S E T G T F Y Q I G D S W E K YCYGRGIGEWHCQPLQTYPSSSGPV 10 T E T P S Q P N S H P I Q W N A P Q P S H I S K Y I L R W RPVSIPPRNLGY

27. A polynucleotide which is capable of hybridising at high stringency to a polynucleotide which encodes the polypeptide whose sequence is

```
NLVATCLPVRASLPHRLN
   MLRGPGPGLLLLAVQCLGTAVPSTGASKSK
   RQAQQMVQPQSPVAVSQSKPGCYDNGKHYQ
   INQQWERTYLGNALVCTCYGGSRGFNCESK
   PEAEETCFDKYTGNTYRVGDTYERPKDSMI
20
   WDCTCIGAGRGRISCTIANRCHEGGQ
   G D T W R R P H E T G G Y M L E C V C L G N G K G E
    I A E K C F D H A A G T S Y V V G E T W E K P
   V D C T C L G E G S G R I T C T S R N R C N D Q
   RIGDTWSKKDNRGNLLQC
                            ICTGNG
25
                          DVRAAVY
    RHTSVQTTSSGSGFF
                         T
   Q P P P Y G H C V T D S G V V Y S V G M Q W L K T
   MLCTCLGNGVSCQETAVTQTYGGN
   V L P F T Y N D R T D S T T S N Y E Q D Q K Y S
   V L V Q T R G G N S N G A L C H F P F L Y N N H N Y
30
   SEGRRDNMKWCGTTQNYDADQKFGFC
   H E E I C T T N E G V M Y R I G D Q W D K Q H D M G H M M R
    TCVGNGRGEWTCIAYSQLRDQCIV.DDITY
    V N D T F H K R H E E G H M L N C T C F G Q G R G R W K C
    PVDQCQDSETGTFYQIGDSWEKYVHG
35
     YCYGRGIGEWHCQPLQTYPSSSGPVEVF
    TETPSQPNSHPIQWNAPQPSHISKYILRW
   RPVSIPPRNLGY
```

- but not to a polynucleotide which encodes a fibronectin which contains the amino acid sequence FYSCTTEGRQDGHLWC.
 - 28. A polynucleotide according to any one of Claims 26 to 27, wherein the polynucleotide is an oligonucleotide.

- 29. A polynucleotide according to any one of Claims 26 to 28, wherein the polynucleotide which encodes a fibronectin or the polynucleotide which encodes the polypeptide as said is a mRNA or a cDNA.
- 5 30. A method of diagnosing cancer the method comprising detecting in a sample from the person to be diagnosed the presence of a polypeptide whose sequence is

```
NLVATCLPVRASLPHRLN
   MLRGPGPGLLLLAVQCLGTAVPSTGASKSK
    Q A Q Q M V Q P Q S P V A V S Q S K P G C Y D N G K H Y Q
10
    NQQWERTYLGNALVCTCYGGSRGFNCE
    EAEETCFDKYTGNTYRVGDTYERPKDS
          IGAGRGRISCTIANRCHEGGQ
       T C
       WRRPHETGGYMLECVCLGNGKGEWT
    I A E K C F D H A A G T S Y V V G E T W E K P Y Q G W M M
15
       TCLGEGSGRITCTSRNRCNDQDTRT
   RIGDTWSKKDNRGNLLQCICTGNGRGEWKC
                  GSGPFTDVRAAVYQPQPHP
   ERHTSVQT
              TSS
                \verb|T| D S G V V Y S V G M Q W L K T Q G N | \\
    PPPYGHCV
                  C Q E T A V T Q T Y G G N S N G
               v s
          LGNG
20
   MLCTC
               TDSTTSNYEQDQKYSFC
   VLPFTYNDR
                  GALCHFPFLYNNHNYTDCT
   VLVQTRGGNSN
                  G T T Q N Y D A D Q K F G F C P M A A
   SEGRRDNMKWC
   H E E I C T T N E G V M Y R I G D Q W D K Q H D M G H M M R
                     IAYSQLRDQCIVDDITY
   CTCVGNGRGEWTC
25
   NVNDTFHKRHEEGHMLNCTCFGQGRGRWKC
   D P V D Q C Q D S E T G T F Y Q I G D S W E K Y V H G V R Y
    CYCYGRGIGEWHCQPLQTYPSSSGPVEVF
   Ī T E T P S Q P N S H P I Q W N A P Q P S H I S K Y I L R W
   RPVSIPPRNLGY
30
```

or a natural variant containing the amino acid sequence VSIPPRNLGY, PCVLPFTYNDRTD, DRTDSTTSNYEQDQ, TDHTVLVQTR and/or REGNSNGALCH or fragment thereof using a reagent which can distinguish said polypeptide from a fibronectin which contains the amino acid sequence FYSCTTEGRQDGHLWC wherein said reagent is an antibody according to any one of Claims 14 to 20.

31. A method of determining susceptibility to cancer the method comprising detecting in a sample derived from the person to be tested the presence of a polypeptide whose sequence is

```
NLVATCLPVRASLPHRLN
  MLRGPGPGLLLLAVQCLGTAVPSTGASKSK
5
  RQAQQMVQPQSPVAVSQSKPGCYDNGKHYQ
  INQQWERTYLGNALVCTCYGGSRGFNCESK
   EAEETCFDKYTGNTYRVGDTYERPKDSMI
  WDCTCIGAGRGRISCTIANRCHEGGQSYKI
   DTWRRPHETGGYMLECVCLGNGKGEWTCK
10
   IAEKCFDHAAGTSYVVGETWEKP
   DCTCLGEGSGRITCTSRNRCNDQDTRT
                         ICTGNGRGEWKC
  RIGDTWSKKDNRGNLL
                       QC
   RHTSVQTTSSGSGFF
                       TDVRAAVYQP
  Q P P P Y G H C V T D S G V V Y S V G M Q W L K T Q G N K Q
15
  MLCTCLGNGVSCQETAVTQTYGGNSÑGE
    LPFTYNDRTDSTTSNYEQDQKYSF
    LVQTRGGNSNGALCHFPFLYNNHNYTDCT
    EGRRDNMKWCGTTQNYDADQKFG
                                 F C
    EEICTTNEGVMYRÏGDQWDKQHDMGHMMR
20
      V G N G R G E W T C I A Y S Q L R D Q C I V D D
     С
      D T F H K R H E E G H M L N C T C F G Q G R G R W
    PVDQCQDSETGTFYQIGDSWEKYVHG
       Y G R G I G E W H C Q P L Q T Y P S S S G P V
     Y C
    T E T P S Q P N S H P I Q W N A P Q P S H I S K Y I L R W
25
   RPVSIPPRNLGY
```

or a natural variant containing the amino acid sequence VSIPPRNLGY, PCVLPFTYNDRTD, DRTDSTTSNYEQDQ, TDHTVLVQTR and/or REGNSNGALCH or fragment thereof using a reagent which can distinguish said polypeptide from a fibronectin which contains the amino acid sequence FYSCTTEGRQDGHLWC wherein said reagent is an antibody according to any one of Claims 14 to 20.

35 32. A method of determining the likely outcome of a patient with cancer the method comprising detecting in a sample from the patient the presence of a polypeptide whose sequence is

N L V A T C L P V R A S L P H R L N

M L R G P G P G L L L L A V Q C L G T A V P S T G A S K S K

40 R Q A Q Q M V Q P Q S P V A V S Q S K P G C Y D N G K H Y Q

I N Q Q W E R T Y L G N A L V C T C Y G G S R G F N C E S K

P E A E E T C F D K Y T G N T Y R V G D T Y E R P K D S M I

WDCTCIGAGRGRISCTIANRCHEGGQSYKI GDTWRRPHETGGYMLECVCLGNGKGEWTCK PIAEKCFDHAAGTSYVVGETWEKPYQGWMM V D C T C L G E G S G R I T C T S R N R C N D Q D T R T S Y RIGDTWSKKDNRGNLLQCICTGNGRGEWKC 5 ERHTSVQTTSSGSGPFTDVRAAVYQPQ P P P Y G H C V T D S G V V Y S V G M Q W L K T Q G N K Q LCTCLGNGVSCQETAVTQTYGGNSNGEPC V L P F T Y N D R T D S T T S N Y E Q D Q K Y S F C V L V Q T R G G N S N G A L C H F P F L Y N N H N Y T D 10 EGRRDNMKWCGTTQNYDADQKFGFCPMAA E E I C T T N E G V M Y R I G D Q W D K Q H D M G H M M R T C V G N G R G E W T C I A Y S Q L R D Q C I V D D I T Y V N D T F H K R H E E G H M L N C T C F G Q G R G R W K C P V D Q C Q D S E T G T F Y Q I G D S W E K Y V H G 15 CYCYGRGIGEWHCQPLQTYPSSSGPVE TETPSQPNSHPIQWNAPQPSHISKYILRW RPVSIPPRNLGY

- or a natural variant containing the amino acid sequence VSIPPRNLGY, PCVLPFTYNDRTD, DRTVSTTSNYEQDQ, TDHTVLVQTR and/or REGNSNGALCH, or fragment thereof using a reagent which can distinguish said polypeptide from a fibronectin which contains the amino acid sequence FYSCTTEGRQDGHLWC wherein said reagent is an antibody according to any one of Claims 14 to 20.
 - 33. A method according to any one of Claims 30 to 32, wherein the reagent which can distinguish said polypeptide from a fibronectin which contains the amino acid sequence FYSCTTEGRQDGHLWC is an antibody according to any one of Claims 14 to 18.
 - 34. A method of diagnosing cancer the method comprising detecting in a sample from the person to be diagnosed a polynucleotide encoding a polypeptide whose sequence is
- 35 N L V A T C L P V R A S L P H R L N

 M L R G P G P G L L L L A V Q C L G T A V P S T G A S K S K R Q A Q Q M V Q P Q S P V A V S Q S K P G C Y D N G K H Y Q I N Q Q W E R T Y L G N A L V C T C Y G G S R G F N C E S K P E A E E T C F D K Y T G N T Y R V G D T Y E R P K D S M I W D C T C I G A G R G R I S C T I A N R C H E G G Q S Y K I

C T C V G N G R G E W T C I A Y S Q L R D Q C I V D D I T Y
N V N D T F H K R H E E G H M L N C T C F G Q G R G R W K C
D P V D Q C Q D S E T G T F Y Q I G D S W E K Y V H G V R Y
Q C Y C Y G R G I G E W H C Q P L Q T Y P S S S G P V E V F
I T E T P S Q P N S H P I Q W N A P Q P S H I S K Y I L R W
R P V S I P P R N L G Y

5

or a natural variant thereof containing the amino acid sequence VSIPPRNLGY, PCVLPFTYNDRTD, DRTVSTTSNYEQDQ, 10 TDHTVLVQTR and/or REGNSNGALCH, using a reagent which can distinguish said polynucleotide from a polynucleotide encoding a fibronectin which contains the amino acid sequence FYSCTTEGRQDGHLWC wherein said reagent is a polynucleotide according to any one of Claims 26 to 29.

15 36. A method of determining the likely outcome of a patient with cancer the method comprising detecting in a sample from the patient the presence of a polynucleotide encoding a polypeptide whose sequence is

NLVATCLPVRASLPHRLN MLRGPGPGLLLLAVQCLGTAVPSTGASKSK V A V S Q S K P G C Y D N G K H Y Q P RQAQQMVQP Q S 20 TCYGGSRGFNCESK ALVC INQQWERT YLG N Y R V G D T Y E R P K D S M I N T PEAEETCFDK Y Т G IANRCHEGGQSYKI DCTCIGAGRG R I s c Т YMLECVCLGNGKGEWTCK DTWRRPHE T G G $\texttt{T} \;\; \texttt{S} \;\; \texttt{Y} \;\; \texttt{V} \;\; \texttt{V} \;\; \texttt{G} \;\; \texttt{E} \;\; \texttt{T} \;\; \texttt{W} \;\; \texttt{E} \;\; \texttt{K} \;\; \texttt{P} \;\; \texttt{Y} \;\; \texttt{Q} \;\; \texttt{G} \;\; \texttt{W} \;\; \texttt{M} \;\; \texttt{M}$ IAEKCFDHAAG 25 TCTSRNRCNDQDTRTSY V D C T C L G E G S G RI ICTGNGRGEWKC IGDTWSKKDNRGNLL Q C TDVRAAVY RHTSVQTTSSG SGPF $\mathsf{G} \ \mathsf{M} \ \mathsf{Q} \ \mathsf{W} \ \mathsf{L} \ \mathsf{K} \ \mathsf{T} \ \mathsf{Q}$ PPPYGHCVTDSGVVY s v YGGNSN MLCTCLGNGVSC T A V Т QT QΕ 30 QKYSFCT Y Q D PFTYNDRTDS Т T S N Ε Y N N H N F Ρ F V L V Q T R G G N S N G A L СН L A D QKFG FCPMAA SEGRRDNMKWCG T Т QN Υ D HDMGHMMR HEEICTTNEGVM Y R I G D Q W D K Q V D D CI TCVGNGRGEWT С IAY S Q L R D Q 35 F QGRGRW NVNDTFHKRHEEG тС G H M L Ν С W E K YV H G P V D Q.C Q D S E T G тF Y Q I G D S YCYGRGIGEWHCQPLQTY SSGPVE ΡS ITETPSQPNSHPIQWNAPQPSHISKYILRW RPVSIPPRNLGY 40

or a natural variant thereof containing the amino acid sequence VSIPPRNLGY, PCVLPFTYNDRTD, DRTVSTTSNYEQDQ,

G D T W R R P H E T G G Y M L E C V C L G N G K G E W T C K IAEKCFDHAAGTSYVVGETWEKPYQGW TCLGEGSGRITCTSRNRCNDQDTRT RIGDTWSKKDNRGNLLQCICTGNGRGEW RHTSVQTTSSGSGPFTDVRAAVYQPQ 5 $\verb"TDSGVVYSVGMQWLKTQGN" \\$ PPPYGHC V SCQETAVTQTYGGNSNG LGNGV T C T T S N Y E Q D Q K Y S F TYNDRTDS ΡF V Q T R G G N S N G A L C H F P F L Y N N H N Y T D C T G R R D N M K W C G T T Q N Y D A D Q K F G F 10 I C T T N E G V M Y R I G D Q W D K Q H D M G H M M R CVGNGRGEWTCIAYSQLRDQCIVDDITY NVNDTFHKRHEEGHMLNCTCFGQGRGRWKC DPVDQCQDSETGTFYQIGDSWEKYVHG YGRGIGEWHCQPLQTYPSSSGPVEVF 15 TETPSQPNSHPIQWNAPQPSHISKYILRW RPVSIPPRNLGY

20

25

or a natural variant thereof containing the amino acid sequence VSIPPRNLGY, PCVLPFTYNDRTD, DRTVSTTSNYEQDQ, TDHTVLVQTR and/or REGNSNGALCH, using a reagent which can distinguish said polynucleotide from a polynucleotide encoding a fibronectin which contains the amino acid sequence FYSCTTEGRQDGHLWC wherein said reagent is a polynucleotide according to any one of Claims 26 to 29.

35. A method of determining susceptibility to cancer the method comprising detecting in a sample derived from the person to be tested the presence of a polynucleotide encoding a polypeptide whose sequence is

```
NLVATCLPVRASLPHRLN
   MLRGPGPGLLLLAVQCLGTAVPSTGASKSK
30
   RQAQQMVQPQSPVAVSQSKPGCYDNGKHYQ
                            YGGSRGFNCESK
                         T C
                  NALVC
   INQQWERTYLG
                        Y
                         RVGD
                              TYERPKDSMI
    EAEETCFDKYT
                   G
                     Ν
                      Т
                          ANRC
                                HEGGQSYKI
    DCTCIGAGRGRISC
                        Т
                         I
                                NGKGEWT
                          VCLG
    DTWRRPHETGGY
                     M L
                        Е
                         С
35
                             TWEKPY
                           GΕ
    IAEKCFDHAAGT
                     S
                      Y
                        V
                         V
                                NDQDTRTSY
                           RNRC
          LGEGSGRITC
                        T S
       T C
                                G N G R
       D T W S K K D N R G N
                            I C T
                      L
                        L
                         Q
                           С
                                   Y
                             RAAV
                                    Q
    RHTSVQTTSSGS
                     G
                      P
                        F
                         T
                           D
                            V
                                   K T
        YGHCVTDSG
                     V V
                        Y S
                           V G
                             M Q M
                                  \Gamma
40
                           T
                              T Y
                                GG
        CLGNGVSCQ
                     ΕT
                        Α
                         V
                            Q
                                   SF
    LPFTYNDRTDSTT
                             D Q
                                K Y
                      S
                        N Y
                           E
                            Q
                                   H N
    LVQTRGGNSNGA
                                Ν
                                  N
                     L
                        H F
                           Р
                            F
                              L
                               Y
                      С
                      QNY
                          DADQKF
                                   GFCPMAA
   SEGRRDNMKWCGTT
   H E E I C T T N E G V M Y R I G D Q W D K Q H D M G H M M R
45
```

TDHTVLVQTR and/or REGNSNGALCH, using a reagent which can distinguish said polynucleotide from a polynucleotide encoding a fibronectin which contains the amino acid sequence FYSCTTEGRQDGHLWC wherein said reagent is a polynucleotide according to any one of Claims 26 to 29.

5

A method according to any one of Claims 34 to 36, wherein the 37. reagent which can distinguish said polynucleotide from a polynucleotide encoding a fibronectin which contains the amino acid sequence FYSCTTEGRQDGHLWC is a polynucleotide according to Claim 28.

10

15

A method according to any one of Claims 30 to 37, wherein the 38. cancer is breast cancer.

39.

Use of a reagent as defined in any one of Claims 30 to 33in the manufacture of a reagent for diagnosing cancer.

Use of a reagent as defined in Claim 39, as a diagnostic agent. 40.

20

A method of modulating cell migration the method comprising 41. administering an effective amount of a polypeptide according to any one of Claims 10 to 13 to the site where modulation of cell migration is required.

A method according to Claim 41, wherein the cell is a fibroblast or an 42. endothelial cell.

25

. A method according to Claim 41 or 42, wherein the site is in a mammalian body.

- 44. A method according to Claim 43, wherein the site is in a human body.
- 45. Use of a polypeptide according to any one of Claims 10 to 13, in the manufacture of an agent for modulating cell migration.
- 46. Use of a polypeptide according to any one of Claims 10 to 13, for modulating cell migration.

5

- 47. A method of healing a wound the method comprising administering to the locality of the wound an effective amount of a polypeptide according to any one of Claims 10 to 13.
 - 48. Use of a polypeptide according to any one of Claims 10 to 13, in the manufacture of a medicament for healing wounds.
- 49. Use of a polypeptide according to any one of Claims 10 to 13, for healing wounds.
- 50. A pharmaceutical composition comprising a polypeptide according to any one of Claims 10 to 13 and a pharmaceutically acceptable carrier.
 - 51. A polypeptide according to any one of Claims 10 to 13 for use in medicine.
- 52. A method of preventing scarring comprising administering to the locality of the site where scarring is to be prevented an effective amount of a polypeptide according to any one of Claims 10 to 13.

HELD

```
Reset
```

Complete entries *:

```
2481 AA.
                    STANDARD;
                                    PRT:
     FINC XENLA
ID
     091740;
AC
     01-NOV-1997 (Rel. 35, Created)
DT
     01-NOV-1997 (Rel. 35, Last sequence update)
DT
     15-JUL-1999 (Rel. 38, Last annotation update)
DT
     FIBRONECTIN PRECURSOR.
DR
GN
     FN1.
     Menopus laevis (African clawed frog).
os
     Bukaryota; Metazoa; Chordata; Craniata; Vertebrata; Buteleostomi;
oc
     Amphibia; Batrachia; Anura; Mesobatrachia; Pipoidea; Pipidae;
OC
     Xenopodinae; Xenopus.
OC
RN
     [1]
     SEQUENCE FROM N.A.
RP
     MEDLINE; 92111942.
RX
     Desimone D.W., Norton P.A., Hynes R.O.;
RA
     Identification and characterization of alternatively spliced
RT
     fibronectin mRNAs expressed in early Xenopus embryos.";
RT
     Dev. Biol. 149:357-369(1992).
RL
     -1- FUNCTION: FIBRONECTINS BIND CELL SURFACES AND VARIOUS COMPOUNDS
CC
         INCLUDING COLLAGEN, FIBRIN, HEPARIN, DNA, AND ACTIN. FIBRONECTINS
CC
         ARE INVOLVED IN CELL ADHESION, CELL MOTILITY, OPSONIZATION, WOUND
CC
         HEALING, AND MAINTENANCE OF CELL SHAPE (BY SIMILARITY).
CC
     -!- SUBUNIT: DIMERS OR MULTIMERS OF ALTERNATIVELY SPLICED VARIANTS,
CC
         CONNECTED BY 2 DISULFIDE BONDS NEAR THE CARBOXYL ENDS (BY
CC
         SIMILARITY).
CC
     -! - ALTERNATIVE PRODUCTS: EACH OF THE "EXTRA DOMAIN" & THE CONNECTING
CC
         STRAND 3 ARE PRESENT IN SOME FORMS OF FIBRONECTIN AND ABSENT IN
CC
         OTHERS. THESE DIFFERENCES ARE DUE TO ALTERNATIVE SPLICING.
CC
         TISSUE SPECIFICITY: IN EARLY XENOPUS EMBRYO, CELLULAR FORMS OF
CC
         FIBRONECTIN PREDOMINATE WHICH INCLUDE BOTH EXTRA DOMAINS. IN
CC
         FIBRONECTIN OF EMBRYONIC AND ADULT LIVER THE CONNECTING STRAND 3
CC
         CAN BE EITHER COMPLETELY EXCLUDED OR INCLUDED.
     -i- SIMILARITY: CONTAINS 12 PIBRONECTIN TYPE I DOMAINS.
CC
     -!- SIMILARITY: CONTAINS 2 FIBRONECTIN TYPE II DOMAINS.
CC
     -!- SIMILARITY: CONTAINS 17 FIBRONECTIN TYPE III DOMAINS.
CC
CC
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CC
     EMBL; M77820; AAA49707.1; -.
DR
      HSSP; P02751; 2FN2.
DR
      PFAM; PF00039; fn1; 12.
DR
      PFAM; PF00040; fn2; 2.
DR
      PFAM; PF00041; fn3; 17.
DR
      PRINTS; PRODO12; PNTYPEI.
DR
      PRINTS; PRODUIZ; PNTYPEII.
DR
      PRINTS; PRO0014; FNTYPEIII.
DR
      PROSITE; PS00022; EGF_1; 2.
DR
      PROSITE; PS01253; FIBRONECTIN_1; 11.
DR
      Glycoprotein; Plasma; Heparin-binding; Acute phase; Cell adhesion;
KW
      Repeat; Alternative splicing; Signal.
KW
                                    POTENTIAL.
FT
      SIGNAL
                    1
                         31
                                    PIBRONECTIN.
PT
                        2481
      CHAIN
                                    FIBRIN- AND HEPARIN-BINDING 1.
                         275
                   55
      DOMAIN
PT
                                    COLLAGEN - BINDING.
                         609
                  309
FT
      DOMAIN
                        1172
                                    BY SIMILARITY.
 PT
      DNA BIND
                  907
                                    CELL-ATTACHMENT.
                 1358
                        1631
 FΤ
      DOMAIN
                        2082
                                    HEPARIN-BINDING 2.
                 1812
PT
      DOMAIN
                                    FIBRIN-BINDING 2.
                 2301
                        2432
 FT
      DOMAIN
                                    PIBRONECTIN TYPE-I 1.
                          93
 PT
      DOMAIN
                   53
                                    FIBRONECTIN TYPE-I 2.
 FT
      DOMAIN
                   98
                         141
                                    FIBRONECTIN TYPE-I 3.
                  142
                         185
 FT
      DOMAIN
                                    FIBRONECTIN TYPE-I 4.
                  187
                         231
 FT
      DOMAIN:
                                    FIBRONECTIN TYPE-I 5.
                  232
                         276
      DOMAIN
 FT
                                    FIBRONECTIN TYPE-I 6.
                  307
                         346
 FT
      DOMAIN
                                    PIBRONECTIN TYPE-II 1.
                         405
      DOMAIN
                  346
 FT
                                    FIBRONECTIN TYPE-II 2.
                  406
                         470
 FT
      DOMAIN
                                    FIBRONECTIN TYPE-I 7.
                         512
      DOMAIN
                  469
 FT
```

```
FIBRONECTIN TYPE-I 8.
                         559
     DOMAIN
                  517
                                    PIBRONECTIN TYPE-I 9.
     DOMAIN
                  560
                          603
                                    PIBRONECTIN TYPE-III 1.
                          707
                  610
   · DOMAIN
FT
                                    FIBRONECTIN TYPE-III 2.
                          809
                  708
     DOMAIN
FT
                                    PIBRONECTIN TYPE-III 3.
                  810
                         904
     DOMAIN
FT
                                     PIBRONECTIN TYPE-III 4.
                  905
                         995
     DOMAIN
FT
                                    FIBRONECTIN TYPE-III 5.
                         1085
                  996
     DOMAIN
FT
                                     PIBRONECTIN TYPE-III 6.
                         1173
                 1086
     DOMAIN
PT
                                     FIBRONECTIN TYPE-III 7.
                 1174
                         1265
     DOMAIN
FT
                                     PIBRONECTIN TYPE-III 8 (EXTRA DOMAIN).
                 1266
                         1356
     DOMAIN
FT
                                     FIBRONECTIN TYPE-III 9.
                 1357
                         1447
PT
     DOMAIN
                                     PIBRONECTIN TYPE-III 10.
                 1448
                         1537
     DOMAIN
FT
                                     FIBRONECTIN TYPE-III 11.
                 1538
                         1631
     DOMAIN
FT
                                     FIBRONECTIN TYPE-III 12.
                         1721
                 1632
     DOMAIN
PT
                                     PIBRONECTIN TYPE-III 13 (EXTRA DOMAIN).
                 1722
                         1811
     DOMAIN
FT
                                     FIBRONECTIN TYPE-III 14.
                         1903
                 1812
FT
     DOMAIN
                                     FIBRONECTIN TYPE-III 15.
                         1992
                 1904
     DOMAIN
PT
                                     FIBRONECTIN TYPE-III 16.
                         2082
PT
     DOMAIN
                 1993
                                     CONNECTING STRAND 3 (CS-3) (V REGION).
                  2083
                         2205
     DOMAIN
FT
                                     FIBRONECTIN TYPE-III 17.
     DOMAIN
                  2206
                         2287
PT
                                     PIBRONECTIN TYPE-I 10.
                         2343
                 2299
FΤ
     DOMAIN
                                     FIBRONECTIN TYPE-I 11.
                         2386
                  2344
     DOMAIN
PΤ
                                     PIBRONECTIN TYPE-I 12.
                         2431
     DOMAIN
                  2388
FT
                                     CELL ATTACHMENT SITE.
                  1615
                         1617
PT
      SITE
                                     BY SIMILARITY.
                           81
                   55
      DISULPID
FT
                                     BY SIMILARITY.
                           90
                    79
 FΤ
      DISULFID
                                     BY SIMILARITY.
                          128
      DISULPID
                   100
FT
                                     BY SIMILARITY.
                   126
                          138
      DISULFID
 FT
                                     BY SIMILARITY.
                          172
                   144
      DISULFID
PT
                                     BY SIMILARITY.
                          182
                   170
 PT
      DISULPID
                                     BY SIMILARITY.
                          218
      DISULFID
                   189
 FT
                                     BY SIMILARITY.
                          228
                   216
      DISULPID
FT
                                     BY SIMILARITY.
      DISULFID
                   234
                          263
 PT
                                     BY SIMILARITY.
                          273
                   261
 PT
      DISULFID
                                     BY SIMILARITY.
                   309
                          336
      DISULFID
                                     BY SIMILARITY.
                          343
                   334
      DISULFID
 FT
                                     BY SIMILARITY.
                          387
                   361
      DISULFID
 FT
                                     BY SIMILARITY.
                   375
                           402
      DISULFID
 \mathbf{FT}
                                     BY SIMILARITY.
                           447
                   421
 FT
      DISULFID
                                     BY SIMILARITY.
                           499
                   471
      DISULFID
 PT
                                     BY SIMILARITY.
                           509
                   497
 PT
      DISULFID
                                     BY SIMILARITY.
                           546
 PT
      DISULFID
                   519
                                     BY SIMILARITY.
                   544
                           556
      DISULFID
 FT
                                     BY SIMILARITY.
                           590
                   562
 PT
      DISULFID
                                     BY SIMILARITY.
                           600
                   588
 FT
      DISULPID
                                      BY SIMILARITY.
                          2330
 FT
      DISULFID
                  2301
                                      BY SIMILARITY
                  2328
                          2340
      DISULFID
 FT
                                      BY SIMILARITY.
                          2373
                  2346
      DISULFID
 PT
                                      BY SIMILARITY.
                  2371
                          2383
 FT
      DISULFID
                          2414
                                      BY SIMILARITY.
                  2390
      DISULFID
 PΤ
                                      BY SIMILARITY.
                          2428
                  2412
 FT
      DISULFID
                                      INTERCHAIN (WITH 2463 OF OTHER CHAIN) (BY
                  2459
                          2459
       DISULFID
 PT
                                      SIMILARITY).
 FT
                                      INTERCHAIN (WITH 2459 OF OTHER CHAIN) (BY
                          2463
                  2463
       DISULFID
 PT
                                      SIMILARITY).
 PT
                                      POTENTIAL.
                           431
       CARBOHYD
                    431
 PT
                                      POTENTIAL.
       CARBOHYD
                    529
                           529
 FT
                                      POTENTIAL.
                    543
                           543
       CARBOHYD
 FT
                                      POTENTIAL.
                    877
                           877
 FT
       CARBOHYD
                          1244
                                      POTENTIAL.
                   1244
       CARBOHYD
  PT
                                      POTENTIAL.
                          1291
                   1291
  FT
       CARBOHYD
                                      POTENTIAL.
       CARBOHYD
                   2202
                          2202
  FT
                   2481 AA; 272678 MW; 7E47DF4F6CE72C93 CRC64;
       SEQUENCE
       MRRGALTGLL LVLCLSVVLR AAPSATSKKR RQAQQQQQQQ VVQPQGTQDN HQKGCYDNGK
       YYQINQQWER TYLGNTLVCT CYGGGRGFNC ESKPESEETC PDKYTGVSYR VGETYERPKD
       NMIWDCTCIG AGRGRISCTI ANRCHEGGQS YKIGDTWRRP HETGGYMLEC VCLGNGKGEW
       TCKPVAERCY DNTAGTSYVV GQTWEKPYQG WMMVDCTCLG EGNGRITCSS KNRCNDQDTK
       TSYRIGDTWS KTDTRGNLLQ CICTGNGRGE WKCERHSSAQ ATGTGSNPIT NIQTALYQPD
       SQLEPYGHCV TDNGVLYSLG MRWLRTQGSK QMLCTCLGNG VSCEBTVATI TFGGNANGEP
       CAIPFTHDGK TYYSCTSEGR QDGKLWCATT SNYDSDKKYS FCTEQLALVQ TRGGNSNGAL
       CNPPPLYNNL NYTDSTSEGR QDSMKWCGTT ANYDADQKPG FCPMAAHEEI CTTNEGVMYR
       VGDQWDKQHD QGHMMRCTCV GNGRGEWSCV AYSQLKDQCI VDGLTYNVNS SFTKLHEEGH
       MMNCTCFGQG RGRWKCDAID QCQDTETRQF YQIGDSWEKH LQGVQYQCYC YGKGIGEWHC
       QPLSTSQAGT GPVQVIITES ANFPTSHPIQ WNAPQASHIK NYILRWKPKL KAGPWKQATI
       PGHLNSYTIS GLKPGILYEG QLISILQYGN REVTTFDFTT TTTIHRHSQT ESGETTPLPP
       LVSISESVTE ITASSFLVSW VSASDTVSGF RVEYELSEDG DEKRYLELPN TATSVNIPDL
       LPGRRYNVNV YQITEEGEKS LILSTTQTTA PDAPPEHNVE NVDDTSIMIK WNKPQAPITG
        YRVVYSPSVE GSSTELNLPS TANSVTLTEL LPGIEYNITI YAVEDSLESV PVFIQQGTTG
       TPQTVIVPSP TDLQLVEVTD VKIIIMWTSP QSEVSGYRVV VKPVSPAGRD VQNLPVNRNT FABVVNLQPG RTYSFEVYAV NRGQESEPLV GEPATKLDAP TDLQFTDVTE STVVIIWIPP
```

ONTICEVILIS	VGQTRGGQPS	OFPENOSVIN	HKLDNLLPGT	KYTVSLVALK	GNQQSASASG
VPSTLEPVGS	TTYTUS MINDS MINDS	עלידע שייי	PRIGEREDAK	P2OGGRAF EC	ATOMOGRA
SGLTPGVEYT	VOTOUTOROCV	PETV	VTPLSPPTNL	RLQPSRDSAT	LTVYWDRSIS
TOTAL TOTAL	TOTOMOVONS	LERRYGPSOT	YCVFENLSPG	ARANARATAN	KKKKKSAPLS
OVERT OR TROOT	ULLAULAALU	TSTDLRWTPL	NSSNIIGYRI	TVVAAGESVP	I I EEF VGP I D
CELL DOSTE OF	GIDYRISLIT	LINGGESAPT	TIIQHTAVPP	PTNLRFTNIG	PDNIRVIWSP
DMCTDI CCVI.	VDVSDVKKPD	DVTRLSLSPS	TNMVVLSNLL	BLIRITARA	
TAICUS PROUT.D	COTCTAPSET	TPNSFTVHWI	APRGPITGYR	TKAOTERGE	RPKRERVPPS
DATE OF THE PARTY	DCCPVI.VCTT	ATMGOORSLP	LAGQQATVSD	ABLDIRALSS	SPNITIISME
	TIME CONCORD	TO THE PROPERTY OF THE PROPERT	SNTATIRGLN	PGVSITITVI	AVTGRGDSPA
WENAPAKIIK	TOVDOPTOMA	VTDIODESIE	VKWSPPPGPV	TGYRVTSVPK	2GCGETT 2CA
TODOOTEVET	VGLQPAVEYV	VSIYSOGENG	ESEPLVETAV	TMIDWLYGUI	FIDAGADSIK
TSEDGIBATE	TRYRVTYSSP	EDGVKELFPA	PEGDDDTABL	HGLKPGTEIT	VSIVALHDDM
POPPLICIOS	TAIPAPTNLQ	PSOVTPSGFS	LSWHAPTVHL	TGYLVKANPK	RKTGPTKEVR
T CDCVA ATTV	TGLMVATKYE	VNVYALKDSL	TSQPLQGLIS	TLDNVSPPRK	PRIODVIETT
USEGAMITE.	TATEMENT DAT	PADGONPIRR	TVDADLRTFT	ITGLQPGTDY	KIATALTNDN
AD CCDUMUMIN	TIPA VDSPSNI.	RPLTTTSNSL	LPTWQPPRAR	ITGYLLRYEK	AGGLIKEHLP
DI DACIONES SIT	LTINILE POTTRY	IIYILAVRNN	MKSEPLVGRK	RTDELPRLVT	Phhbcochri
TUNDADERMI	PHITOTKLDN	GNGIQLPGSN	GOOPSSDHEG	OLIERHGERS	PLAPTTAVPV
DDCWFAPGRY	POERVDIELD	TPPVQHGDFD	GPYPHGLGPQ	TWDSGAGRAY	SHITISMEE
T.PTTTRVTTSC	HPIDHKEAPL	OFRVPGTSSS	ATLNGLTRGA	TYNIVVEAQK	GTDKHKVLEK
PUTUGSPGSP	EGVLQPVEDT	CYDTFSGAHY	SVGQEWERMS		LGYGSGHFRC
	TANDOT CERMIN	PROPRICOMMS	CTCLGNGKGE		YDEGKMYNVG
ROWOKRYLGA	ICSCTCYGGO	QGWRCDNCRR	PGAVSPDGTA	. GQTVSQFTQR	YQQNYNLNCP
TRCYLPLGLO	ADTOHSOOTO	K			

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error

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